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Effect of late pregnancy management on behaviour, welfare and calf health in dairy cattle

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Thesis submitted for the degree of Doctor of Philosophy

The University of Edinburgh

2018

Declaration

I declare that I have composed the present thesis. The work described is my own and all assistance received is acknowledged. The work has not been submitted for any other degree or professional qualification.

Mayumi Fujiwara
May 2018

Acknowledgement

I am thankful for all those who have been involved in my PhD journey over the past four years. It has definitely been one of the most challenging periods of my life, but I was able to overcome difficulties thanks to the help of numerous people. I am very fortunate to have three excellent supervisors, Dr Kenny Rutherford, Dr Marie Haskell and Dr Alastair Macrae, who were always willing to share their extensive knowledge and offer valuable feedback on my work (including English language corrections). I was always more motivated after our regular meetings, which sometimes lasted more than two hours. I believe that I would never have finished without their guidance and encouragement.

My sincere gratitude goes to SRUC for their financial support in this PhD. This funding made it possible to pursue my dream of doing PhD abroad. I would also like to thank Dr Sarah Hall and Jennifer Coe for their support on the physiological analyses in the Biomarker's Lab, and the technicians from the SRUC Animal Behaviour and Welfare team for their technical support. Liz Burrough at The Dairy Herd Health and Productivity Service is also acknowledged for conducting the biochemical analyses of blood samples. I thank Dr Helen Brown for her incredible support in every aspect of the statistics used in this thesis.

I would like to acknowledge and thank Dr Jenny Gibbons at AHDB Dairy and George Jamieson at NFU Scotland for their advice on developing the questionnaire, and the technical staff at the Crichton Royal Farm, and Wilson Lee from RDSV Langhill Farm for their help with piloting the survey. I am very thankful to the Royal Association of British Dairy Farmers (RABDF), the Organic Research Centre, Myerscough College, Shepton Vets, SAC Consulting and the Dairy Herd Health and Productivity Service for advertising the survey in their newsletters. I am particularly grateful to the RABDF for their help in distributing the survey to their members, and all the farmers who spared their precious time to participate the survey.

It would not have been possible to complete data collection for the two experiments without the help of the technical staff at the Dairy Research and Innovation Centre and the farm staff at Crichton Royal Farm. Ainsley Bagnall, John Dickinson, Hannah Dykes, and Isla McCubbin are acknowledged for their help in data collection. I wish to thank David Bell for his tremendous support in technical issues and data collection even after the completion of the experiments. Hugh McClymont and Peter Little are also acknowledged for their flexibility in adjusting the management routines. Cows and calves who were unknowingly involved in the two experiments are also greatly appreciated. I also would like to thank Dr Paul Hargreaves for providing me with information relating to the out-wintering project.

I wish to express my warm and sincere thanks to my friends and colleagues at SRUC. My PhD would not have been the same without their support and inspirations. Many thanks to Akke Kok, Jenna Bowen and Virgilio Ambriz-Vilchis for reading portions of my thesis and providing some useful insights. I owe a great deal of thanks to Rhea Kyriazopoulou and Alan Cahill for their assistance with the English language, which was probably the hardest part of writing the thesis.

Finally, I would like to extend my eternal gratitude to my family for always believing in me and providing emotional support as I embarked on this incredible journey.

Conference proceedings

Fujiwara, M., Ensor, H.M., Haskell, M.J., Macrae, A.I., Rutherford, K.M.D. (2016). Effect of overstocking during the non-lactating dry period on the behaviour and welfare of dairy cows. **Poster presentation.** 50th ISAE congress, Edinburgh, United Kingdom, July 2016.

Fujiwara, M., Ensor, H.M., Haskell, M.J., Macrae, A.I., Rutherford, K.M.D. (2016). Effect of overstocking during the non-lactating dry period on the behaviour and welfare of dairy cows. **Poster presentation.** 67th EAAP annual meeting, Belfast, United Kingdom, August 2016.

Fujiwara, M., Haskell, M.J., Macrae, A.I., Rutherford, K.M.D., (2017). Effect of overstocking during the non-lactating (dry) period on behaviour and faecal glucocorticoid metabolites in pregnant dairy cows, and on calf birthweight and growth. **Poster presentation.** British Society of Animal Science, Annual conference, April 2017.

Fujiwara, M., Haskell, M.J., Macrae, A.I., Rutherford, K.M.D., (2017). Effect of overstocking during dry period on behaviour and stress level of dry cows, and on pre-weaning calf growth, accepted for poster presentation. **Poster presentation.** 51st ISAE congress, Aarhus, Denmark August 2017.

Fujiwara, M., Haskell, M.J., Macrae, A.I., Rutherford, K.M.D., Bell, D.J., Hargreaves, P., (2017). Effects of maternal out-wintering on spring born calf's growth, activity levels and learning ability, accepted for theatre presentation. **Oral presentation.** 68th EAAP annual meeting, Tallinn, Estonia, August 2017.

Abstract

Studies in various animal species have demonstrated that stress during pregnancy can have a detrimental effect on progeny health and development throughout its postnatal life. If this were to apply to dairy cows, minimising stress in pregnant cows could be of critical importance in ensuring offspring health and welfare. However, relationships between maternal health and welfare and offspring performance have not been well investigated in dairy cattle. Traditionally management strategies for cows in late pregnancy (i.e. in the dry period) have focused on maximising milk production whilst minimising impacts on postpartum health. This may include management practices that can have a negative impact on the health and welfare of cows in the dry period itself. This project aimed to identify potential sources of stress associated with management practices in late pregnant cows, and to investigate its effects on offspring health, behaviour and welfare.

A survey was conducted to investigate typical dry cow and pre-weaned calf management practices on dairy farms in the United Kingdom (UK). Data from 148 commercial UK dairy farms provided valuable information on dry cow and pre-weaning calf management. Procedures that were commonly practised but potentially stressful for dry cows included the abrupt cessation of milking and frequent changes in diet and social environment.

Two experimental studies were conducted to investigate the impact of alternative management practices in late pregnancy on progeny welfare. The first experiment investigated the behavioural, physiological and metabolic responses of dry cows to industry minimum standards (H: high stocking group) compared to a more extensive space allowance (L: low stocking density). The offspring of these cows were monitored until weaning to assess their responses to typical dairy farm procedures. The second experiment was conducted on calves born to heifers from an out-wintering project. Pregnant heifers were kept either indoor or outdoor grazing (on deferred grass or kale) throughout the winter period. The health, growth and behaviour of offspring were monitored for the first 14 days of life.

Limited feed-face space resulted in altered feeding patterns and increased competition at the feed-face. There was no association between dry period stocking density and the physiology and metabolism of dry cows. Maternal treatment had no impact on pre-weaned calf birth weight, health, growth, passive immunity, neonatal vigour and the majority of behavioural outcomes. However, H calves made more frequent social contact with companions compared to L calves and showed higher behavioural reactivity to weaning. Maternal high stocking density treatment and previous disease incidence in calves reduced the behavioural reactions

to disbudding and the expression of pain-related behaviours. Out-wintering of pregnant heifers on kale showed no negative impact on growth compared to the indoor group, whilst out-wintering on deferred grass resulted in the lowest growth rate. However, out-wintering on deferred grass may have enhanced offspring social motivation and learning ability.

This study has demonstrated potential associations between maternal experience during pregnancy, and offspring growth and behaviour. The effect of maternal treatment on offspring behaviour may be more likely to emerge in challenging situations. Further research will be needed to understand the underlying mechanisms and to reach definite conclusions, which would have implications for improving the welfare of late pregnant cows and their offspring.

Lay summary

Studies in various animal species have demonstrated that stress during pregnancy can have a detrimental effect on the animal throughout its postnatal life. If such effects apply to dairy cows, minimising stress in pregnant cows could be of critical importance in ensuring the health and the welfare of their offspring.

A survey was conducted to investigate the management of pregnant, non-lactating cows and calves prior to weaning on United Kingdom (UK) dairy farms. Data from 148 UK dairy farms showed that potentially stressful procedures for dry cows such as the abrupt cessation of milking, and frequent changes in diet and social environment are commonly practised.

Two experimental studies were conducted to investigate the impact of management practices in late pregnancy on progeny welfare. The first experiment investigated the effect of a high stocking density during late pregnancy on the behavioural, physiological and metabolic responses of cows. The offspring of these cows were monitored until weaning to assess their responses to typical dairy farm procedures.

High stocking density during late pregnancy affected feeding and social behaviour of the cows, but no effects on cow physiology and metabolism or pre-weaned calf body weight, growth and neonatal vigour were found. However, associations between maternal high stocking density and calf's social behaviour, reactions to weaning and a painful procedure (disbudding) potentially exist.

The second experiment was conducted on calves born to pregnant heifers kept either indoors, or outdoor grazing (on deferred grass or kale) throughout the winter period. Their offspring were monitored for the first 14 days of life. Out-wintering on deferred grass resulted in the lowest growth rate, whilst out-wintering on kale showed the similar growth rate to the indoor group.

This study has demonstrated potential associations between maternal experience during pregnancy and offspring growth and behaviour.

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List of abbreviations

Commonly used abbreviations are listed below. Abbreviations that are specific to this thesis are defined within each chapter.

ADG	Average dairy weight gain
AHDB	Agriculture and Horticulture Development Board
AIAO	All-in-all-out
BCS	Body condition score
BHB	ß-hydroxybutyric acid
BW	Body weight
CSV	Comma separated values
CV	Coefficient of variability
DEFRA	Department for Environment, Food and Rural Affairs
df	Degrees of freedom
DHEA	Dehydroepiandrosterone
DM	Dry matter
DMI	Dry matter intake
DOA	Dioxoandrostane
FGCM	Faecal glucocorticoid metabolites
GLM	General Linear Model
GLMM	Generalised Linear Mixed Model
HF	Holstein Friesian
HPA	Hypothalamic-pituitary-adrenal
HR	Hazard Ratio
IMI	Intramammary infections
IQR	Interquartile ranges
LA	Local anaesthesia
LCT	Lower critical temperature
NEFA	Nonesterified fatty acids
NRC	National Research Council
OR	Odds Ratio
RABDF	Royal Association of British Dairy Farmers
REML	Residual Maximum Likelihood
SD	Standard deviation
SEM	Standard errors of means
SRUC	Scotland’s Rural College
THI	Thermal humidity index
TMR	Total mixed ration
TRD	Traditional

Chapter 1 :

Introduction

1.1. Introduction

It is widely accepted that the non-lactating interval prior to calving (i.e. the dry period) is necessary to maximise milk production during the subsequent lactation period (Pezeshki et al., 2010). The dry period was introduced to the dairy industry in the 1800s (Arnold and Becker, 1936), and drying-off approximately 60 days before the expected calving date has been standard practice for decades to optimise subsequent milk production (Bachman and Schairer, 2003). Producers tend to pay less attention to cows in the dry period due to a lack of immediate contribution to profit (Grummer, 1995; Dewhurst et al., 2000). However, in the late 20th century, associations between prepartum energy intake and postpartum health and milk production had been reported (Curtis et al., 1985; Grummer, 1995), and the critical importance of management during the dry period has become well recognised. Since then, prepartum dairy cow management has attracted significant attention from researchers (e.g. nutritional management: Goff, 2006; Ingvartsen, 2006).

Along with research focusing on prepartum dairy cow management for improved postpartum health and productivity (Drackley, 1999; LeBlanc, 2010), there has been concern regarding the effects of traditional dry cow management practices on the health and welfare of cows during the dry period (e.g. Zobel et al., 2015). Improved nutritional management and genetic selection for high yielding traits have dramatically increased the level of milk production of dairy cows in recent decades. However, the length of the dry period and the method of dry-off have not been changed since the beginning of the 20th century (Bachman and Schairer, 2003). This raises the question of whether the length of the dry period is suitable for cows in the 21st century (Grummer and Rastani, 2004; Pezeshki et al., 2010; Van Knegsel et al., 2013), and whether the conventional method of dry-off is appropriate for high yielding cows (Zobel et al., 2015).

Proudfoot and Habing (2015) recently highlighted the association between social stress and increased disease risks in farm animals, and suggested that some management practices could be a source of social stress. For example, cows in the late gestation period often experience frequent regrouping from around dry-off until calving as a part of management practices (Cook and Nordlund, 2004), which would disrupt social hierarchy. In intensive farming systems, cows are subject to high stocking rates (Estevez et al., 2007), as a certain level of overstocking is believed to increase profit per unit (De Vries et al., 2016). However, overstocking increases competition for resources (e.g. Huzzey et al., 2012; Proudfoot et al., 2009). Other management factors such as housing type and atmospheric temperature could also affect the behaviour, health and welfare of dairy cows (Tao and Dahl, 2013; de Vries et al., 2015).

The health and welfare of cows during the dry period can be an important determinant of their postpartum performance (e.g. Huzzey et al., 2011), and can be partly influenced by management practices. Moreover, it has been reported in mammalian species that maternal health and welfare can have a critical impact on fetal development and offspring performance throughout postnatal life (Weinstock, 2008; Rutherford et al., 2012). This would suggest that there is the potential that stressful experiences of cows during the dry period could not only affect their postpartum performance, but also the health and productivity of their offspring. This would consequently affect future profitability of the farm. The aim of this review is to summarise the existing literature related to dry cow management, and to identify potential sources of stress for cows associated with typical dry cow management practices. Additionally, the potential effects of maternal health and stress status on offspring performance are discussed.

1.2. Dry off procedure

1.2.1. Difference between natural (gradual) and abrupt dry-off

Under natural conditions, milk production in mammals gradually decreases as infants reduce the frequency of suckling while increasing the intake of solid feed. Residual milk in the udder enhances the regression of the mammary gland which is mediated by a change in circulating hormones (Monks et al., 2002). Gradual involution of the mammary gland is preceded by metabolic and immunological responses that protect the mammary gland from new infection (Silanikove et al., 2013). However, the milk production of dairy cows on farms is maintained until late pregnancy by continuous removal of milk from the udder (i.e. milking), and milking is rather abruptly ceased around 60 days prior to calving. The abrupt cessation of milking leads to an acute involution of the mammary gland, which is rarely seen under natural conditions (Zobel et al., 2015).

The improved nutritional management and genetic selection for milk yield have enabled dairy cows to produce greater amounts of milk at dry-off, compared to when the abrupt dry-off procedure was first established on commercial dairy farms (Zobel et al., 2015). The process of acute involution of the mammary gland in high yielding cows resembles the wound healing process (Silanikove et al., 2013). This unnatural process of involution interferes with natural metabolic and immunological adaptations in the mammary gland, leaving the mammary gland susceptible to infections (Silanikove et al., 2013). Indeed, it has been reported that cows are at the highest risk of udder infection around the dry period

(Bradley and Green, 2004). and this is most applicable to high yielding cows (Dingwell et al., 2002; Rajala-Schultz et al., 2005; Newman et al., 2010). The delayed development of the mammary defence system due to forced dry-off may partly contribute to the increased risks of intramammary infections (IMI) at dry-off (Dingwell et al., 2001).

1.2.2. Dry cow therapy

Infusion of long-acting antibiotics into the udder at dry-off (i.e. antibiotic dry cow therapy) is an established method to prevent the introduction of new IMI, or treat existing IMI caused by gram-positive bacteria (Berry and Hillerton, 2002a). Dry cow therapy successfully reduced the risks of new IMI when used in combination with internal or external teat sealants (Berry and Hillerton, 2002b; Bradley et al., 2011). However, Zobel et al. (2015) discussed the limitations of this method in their review. They argued that the efficacy of antibiotics gradually disappears over the dry period (e.g. Pinedo et al., 2012), and the antibiotics used in this therapy did not target infections caused by gram-negative bacteria. Moreover, the widespread use of antibiotics is becoming a public concern (Barkema et al., 2015). Some studies have suggested the use of teat sealants without antibiotics as a potential alternative to dry cow therapy (Huxley et al., 2002; Bhutto et al., 2011), although the application of teat sealant alone cannot treat existing IMI.

1.2.3. High milk production at dry-off

1.2.3.1. Risk factors for new intramammary infection (IMI) at dry-off

There are various factors and mechanisms involved in the increased risk of new IMI during the dry period. A relatively old study reported that incomplete teat closure and milk leakage after dry-off was associated with the increased likelihood of clinical mastitis during the dry period (Schukken et al., 1993). More recent studies have reported that higher milk production at dry-off was associated with a higher incidence of milk leakage after dry-off (Tucker et al., 2009; Bertulat et al., 2013). Cows with higher milk production (>20 kg/day) at dry-off were five times more likely to have milk leakage from their teats compared to cows with lower milk production (<15 kg/day) at dry-off (Bertulat et al., 2013). Dingwell et al. (2004) found that higher milk production before dry-off (>21 kg) was associated with delayed teat closure after dry-off, and that cows with open teats during the dry period were more likely to develop new IMI at calving. These studies suggest that open teats can be a common route of bacterial infection into the udder during the dry period, and that high milk production at dry-off can increase the chance of infection.

1.2.3.2. Udder discomfort

Bertulat et al. (2013) found that high milk production at dry-off was associated with increased udder pressure. This study also measured the concentrations of faecal cortisol metabolites of cows with three different production levels at dry-off (low: <15 kg/d, medium: 15-20 kg/d, and high: >20 kg/d). Increased pressure in the udder was observed soon after dry-off, and was followed by an increase in faecal glucocorticoid metabolites. There was a positive correlation between udder pressure and levels of faecal glucocorticoid metabolites on the following day after dry-off. Moreover, the increase in the level of faecal glucocorticoid metabolites of mid to high yielding cows (at dry-off) was greater than low yielding cows. The concentration of faecal glucocorticoid metabolites remained significantly higher than baseline until nine days after dry-off. However, the significant increase in the concentration of glucocorticoid metabolites of low yielding cows was observed for only three days following dry-off.

Cows in the study by Bertulat et al. (2013) were abruptly dried-off, but an increase in the plasma cortisol level was also observed in cows that were gradually dried-off (Odensten et al., 2007a). This study investigated plasma cortisol responses of cows with three different production levels at dry-off (low: 5.0-11.4 kg/day, medium: 11.5-17.7 kg/day, high: 17.8-29.5 kg/day) to a gradual dry-off procedure (over five days with milking once per day on d2 and d5). The increase in plasma cortisol was observed in the medium and high yielding cows but not in the low yielding cows, and the level of increase was greatest in high yielding cows. The results from these two studies suggest that cows with high milk production at dry-off can experience stress and udder discomfort at dry-off, regardless of the dry-off method.

1.2.4. Management practices to reduce milk production at dry-off

Compared to the amount of research and knowledge invested in improving mammary health during the dry period, research on the effect of common dry-off procedures on cow welfare is limited to biological functioning and health (Zobel et al., 2015). However, some studies have reported alternative dry-off methods that can reduce milk production at dry-off, which would reduce distress to cows in this period.

1.2.4.1. Reduction in milking frequency

Intermittent milking (i.e. reducing the frequency of milking over the days before dry-off) is recommended as an alternative to abrupt dry-off. Compared to the abrupt cessation of the regular milking schedule (e.g. milking twice daily), once daily milking over seven days (Tucker et al., 2009) or a gradual reduction in the milking frequency over five to seven days (Newman et al., 2010; Zobel et al., 2013) successfully reduced milk production at dry-off (Tucker et al., 2009; Newman et al., 2010; Zobel et al., 2013), the likelihood of milk leakage after dry-off (Zobel et al., 2013) and the probability of new IMI (Newman et al., 2010).

1.2.4.2. Feed restriction

A reduction in milk production can be also achieved by feed restriction. Tucker et al. (2009) reported that offering cows 8 kg Dry Matter (DM) silage over seven days along with intermittent milking reduced milk production by 34% compared to cows fed 16 kg DM silage. Cows fed 16 kg DM silage showed a higher incidence of milk leakage and more cases of subclinical mastitis compared to cows fed 8 kg DM silage after dry-off. Moreover, cows fed 16 kg DM silage had firmer udders and spent less time lying. Although the shorter lying time could result from the different time budget (16 kg DM cows spent more time feeding), the more frequent lateral lying pattern observed in the cows fed 16 kg DM could indicate that those cows experienced udder discomfort due to increased firmness. However, cows fed 8 kg DM silage vocalised three times more than cows fed 16 kg DM silage during and after the dry-off, suggesting that cows fed 8 kg DM were hungry.

Feed restriction can be categorised as either quantitative or qualitative restriction. Quantitative restriction attempts to reduce the amount of diet consumed by individual animals (D'Eath et al., 2009), but this system may be difficult to achieve in a loose housing group system. It can result in an imbalance in energy intake between dominant and subordinate cows (e.g. Olofsson, 1999). Moreover, quantitative restriction has been reported to increase foraging activities, indicating hunger (Lawrence et al., 1993). Qualitative restriction reduces the energy density of the diet whilst increasing the amount of diet (D'Eath et al., 2009). Such a diet aims to promote satiety by increasing gut-fill with high fibre, and is often offered *ad libitum*. However the *ad libitum* intake of a low quality diet does not necessarily provide sufficient nutrients for animals (D'Eath et al., 2009).

The change in milk production and metabolism of cows which were offered two different qualities of diet (straw only *ad libitum,* or straw *ad libitum* and 4kg DM silage) during dry-off (over five days with intermittent milking) were investigated by Odensten et al. (2005) and Odensten et al. (2007b). These two studies found that both dietary management techniques successfully reduced milk production at dry-off with no difference in the level of reduction. After the diet was switched from the lactating ration to the dry-off diets, significantly higher concentrations of plasma cortisol were observed in cows fed straw only, and the concentrations were significantly higher than cows fed silage and straw during dry-off (Odensten et al., 2007b).

Cows fed straw only showed greater negative energy balance and higher rumen pH with low volatile fatty acids during dry-off compared with late lactation (Odensten et al., 2005). The effects of this dietary change on the rumen environment and metabolism observed in cows fed silage and straw were not as severe as cows fed straw only. These results suggest that the dietary change from lactating ration to straw only can be distressing to cows, changing the rumen conditions. In contrast, the addition of silage to straw could mitigate the negative effect of a sudden dietary change. These studies did not record behavioural changes in cows, so it is unclear whether cows fed straw only experienced excessive hunger, and if 4 kg DM silage was enough to satiate animals.

1.2.5. Addressing welfare problems associated with feed restriction

Feed restriction in farm animals is a serious welfare concern, as animals suffer from hunger and insufficient energy intake. Therefore, it is important to balance the positive effects of reduced milk production at dry-off (removal of udder discomfort or pain) and the negative effect of feed restriction. A study by Valizaheh et al. (2008) suggested a potential alternative method to achieve a reduction in milk production without inducing excessive hunger in cows. In this study, they offered two diets with different digestibility to cows around dry-off (intermittent milking for six days), and investigated the effect on milk production and behaviour. Cows were initially offered a total mixed ration (TMR), but when the dry-off procedure started, they were fed either oat hay (containing more fibre and less protein) or grass hay (containing less fibre and more protein) *ad libitum* for six days. Regardless of the treatment group, cows decreased milk production, but the decrease in milk production was greater in the group fed oat hay.

Although the udder size was not different between the two groups, cows fed oat hay spent more time standing inactive (not feeding) and less time lying. DM intake (DMI) also decreased for both groups after being switched from the TMR, and cows fed oat hay had a significantly lower energy intake compared to cows fed grass hay. Increased vocalisations were observed after the dietary change in both of the dietary groups, but cows fed oat hay vocalised more than cows fed grass hay. The authors concluded that replacing TMR with oat or grass hay caused frustration and hunger in cows, and cows fed oat hay were more severely affected. In their review, Zobel et al. (2015) discussed that the higher palatability and higher levels of protein in grass hay may have promoted satiety in cows fed grass hay.

1.2.6. Summary of this section

It appears from the studies described above that abrupt dry-off may not be appropriate for high yielding cows, and that efforts to reduce milk production prior to dry-off would be warranted. The studies above also showed that it is possible to manage milk production at dry-off, but feed restriction could be more distressing to cows compared to intermittent milking. As Zobel et al. (2015) pointed out, there is a lack of research on the behavioural responses of cows to dry-off procedures. Further research is required to investigate optimal strategies to reduce milk production at dry-off for high yielding cows, whilst minimising the negative impacts on udder health and discomfort.

1.3. Nutritional management for dry cows

Prepartum nutritional management of cows is of critical importance in determining postpartum performance. It has been reported that cows decrease DMI by approximately 30% in the two to three weeks prior to parturition (Bertics et al., 1992; Grummer, 1995), and prepartum feed intake was reflected in postpartum feed intake, health and milk production (Grummer, 1995). More recent studies have shown that prepartum negative energy balance (Ospina et al., 2010a; Chapinal et al., 2011; Huzzey et al., 2011), prepartum lower DMI (Huzzey et al., 2007; Goldhawk et al., 2009), and a reduction in prepartum feeding time (Urton et al., 2005) are associated with increased risks of postpartum disorders. Moreover, prepartum negative energy balance has been associated with loss of milk production during the subsequent lactation (Ospina et al., 2010b; Chapinal et al., 2012). Since most of the disease incidence in dairy cows are concentrated in the first month after parturition (LeBlanc et al., 2006), correct nutritional management before parturition is essential to maintain the health and productivity of dairy cows.

1.3.1. High energy diet for dry cows

There are studies that have investigated the effect of higher prepartum energy intake on DMI, energy status and production (e.g. Dann et al., 1999; Doepel et al., 2002; Rabelo et al., 2003; Vandehaar et al., 1999). Feeding a diet containing highly digestible carbohydrates improved prepartum DMI and metabolic status (Dann et al., 1999). Offering a diet with high energy and protein to cows during the last 25 days (Vandehaar et al., 1999) or the last 21 days (Doepel et al., 2002) prepartum did not affect prepartum DMI, but improved energy balance was observed in prepartum cows. Rabelo et al. (2003) only investigated postpartum performance, and found that feeding a diet with a high energy density during the last 28 days prepartum improved feed intake and energy balance. However, these positive effects were mainly observed in multiparous cows.

The studies described in the previous paragraph modified the diet during the last month before calving. However, Dann et al. (2006) reported that the energy levels of a diet during the close-up dry period (from -24 days relative to calving) had little effect on postpartum performance, compared to plane of nutrition during the far-off dry period (from dry-off to

-25 days prepartum). This study found that overfeeding of cows during the far-off dry period resulted in compromised prepartum energy status and decreased postpartum DMI irrespective of the diet during the close-up dry period. Therefore, the authors concluded that overfeeding during the far-off dry period can be detrimental to prepartum energy status and postpartum performance.

1.3.2. Energy restriction for dry cows

Nutritional requirements for non-lactating cows are normally lower compared to lactating cows (Quigley and Drewry, 1998), so a diet containing the equivalent energy level as lactating cows would lead to excessive body weight gain during the dry period. Grummer (1995) pointed out that cows that were over-conditioned at calving were more likely to have lower postpartum DMI. Additionally, overfeeding of dry cows and high body condition score (BCS) at calving have been associated with higher incidence of dystocia and metabolic disorders, and lower milk production after parturition (Grummer, 1993; Rukkwamsuk et al., 1998).

Therefore, some studies have investigated the effect of feed restriction during the dry period (Agenäs et al., 2003; Douglas et al., 2006; Holtenius et al., 2003). Agenäs et al. (2003) and Holtenius et al. (2003) offered three different amounts of the same diet (Low: 6kg DM ration and 71MJ/d; Moderate: 9kg DM and 106 MJ/d; High: 14.5 kg DM and 177 MJ/d) during the eight weeks prepartum. These studies found that prepartum quantitative feed restriction resulted in improved postpartum DMI (Agenäs et al., 2003; Holtenius et al., 2003) and an improved postpartum energy balance (Holtenius et al., 2003). Similarly, Douglas et al. (2006) reported that feed restriction (80% of Net Energy of lactation diet) during the 60 days prepartum resulted in an improved postpartum energy balance and a lower incidence of displaced abomasum, compared to an *ad libitum* intake group (160% of Net Energy of lactating diet) during the same period.

However, Winkelman et al. (2008) did not find a clear improvement in postpartum energy status in cows with restricted feeding during the 45 days prepartum. Similarly, Holcomb et al. (2001) did not find any improvement in postpartum DMI in cows that were feed restricted in the 28 days prepartum, although this study found a better postpartum energy balance in feed restricted cows. Indeed, all of the studies described above found no positive effect of prepartum feed restriction on milk production. Therefore, prepartum feed restriction may not improve postpartum productivity, especially when the period of feed restriction is shorter.

1.3.3. Problems associated with feed restriction

Feed restriction in farm animals is common (e.g. broiler breeders and dry sows), and it is widely accepted as a serious welfare issue that needs to be addressed, especially when it is chronic (Bokkers et al., 2004). Chronic feed restriction can result in prolonged hunger in animals as their motivation for food remains unsatisfied (see D'Eath et al. 2009). A state of hunger in animals can be measured by behavioural changes, for example an increased frequency of vocalisation (Haley et al., 2005; Valizaheh et al., 2008), feeder visits (de Paula Vieira et al., 2008) and feeding speed (D'Eath et al., 2009). It was reported that wild animals spend less time on resting, body maintenance and affiliative behaviour when they are hungry, and spend more time on foraging (Loy, 1970; Savory and Lariviere, 2000; Hocking et al., 2004).

The studies on feed restriction for prepartum cows described above do not address behavioural changes or welfare problems associated with feed restriction. However, the results from these studies indicate the possibility that cows may have experienced hunger. In the studies by Agenäs et al. (2003) and Holtenius et al. (2003), feed restriction during the dry period resulted in lower body weight gain and loss of body condition score at calving. Douglas et al. (2006) reported that cows offered a ration *ad libitum* during the dry period were capable of consuming 2.2 % of their body weight (14.6 kg DM/d), which was almost double the amount of the feed restricted cows (7.4 kg DM/d). This resulted in feed restricted cows continuously losing body condition and suffering from negative energy balance during the dry period. Since there was no difference in calf birth weight between the dietary treatments, it is possible that maternal tissue could have been mobilised in feed restricted cows to support the growing fetus.

Janovick and Drackley (2010) suggested diluting the energy levels of the diet by adding chopped wheat straw. They adjusted the diet for 100% of National Research Council (NRC) energy requirements, and fed this diet *ad libitum* throughout the dry period, which prevented prepartum over-conditioning compared to a moderate energy density feed *ad libitum* (150% NRC energy requirement). This feeding method (*ad libitum* provision of high forage diet) could also increase the satiety of cows and reduce the variation in energy intake between dominant and subordinate cows in a loose housing group system. Vickers et al. (2013) also compared cows offered either a high forage diet (87%) throughout the dry period or a low forage diet (77%) during the last three weeks before parturition. The prepartum low forage diet increased milk production during the 7-28 days postpartum and postpartum DMI was not different between the two dietary treatments. However, improved postpartum metabolic status and lower incidence of ketosis were observed for cows fed the high forage diet throughout the dry period.

Various studies have described methods to improve the energy status of cows during the pre- and postpartum period. Restricting energy intake for far-off dry cows may be important to prevent over-conditioning and negative energy balance, but also maximising feed intake of close-up cows is essential to prevent postpartum disorders. There are studies suggesting the potential benefits of *ad libitum* intake of a single type of high fibre diet during the entire dry period (Janovick and Drackley, 2010; Vickers et al., 2013). This could simplify the dietary management of dry cows, and could achieve energy restriction without causing severe hunger.

1.3.4. Factors affecting energy intake of individual cows

It is important for all cows to consume a formulated diet of adequate quality and quantity in order to achieve the nutritional management goal i.e. to optimise postpartum DMI, health and milk production. However, individual feed intake can be affected by various factors such as feed bunk management and social environment (Devries and von Keyserlingk, 2008). For example, the delivery of fresh feed stimulates the feeding behaviour of cows (DeVries and von Keyserlingk, 2005), and frequent feed delivery allows cows to have more equal access to feed (DeVries et al., 2005).

Cows typically sort feed for highly palatable components such as grain concentrates and sort against high fibre forage particles (Leonardi and Armentano, 2003; DeVries et al., 2005; Hosseinkhani et al., 2008). This leads to a change in feed quality over time from fresh feed delivery, and an unbalanced distribution of feed if not all cows can access the feed-face simultaneously. Feed-sorting by dominant cows can increase their risk for developing subacute ruminal acidosis as they normally select for small particles, which are higher in energy and protein (Stone, 2004; Krause and Oetzel, 2006). In contrast, subordinate cows may suffer from lower energy intake as only high fibrous feed is available at the feed-face after the feed has been sorted against longer particles (DeVries et al., 2005; Hosseinkhani et al., 2008; Proudfoot et al., 2009).

Overstocking of the feed-face and an associated increase in competition at the feed-face alters the feeding behaviour of cows, including an increased feeding rate (Hosseinkhani et al., 2008; Collings et al., 2011) and reduced peak-time feeding activity (Collings et al., 2011; Huzzey et al., 2012). Some degree of competition for feed is inevitable in a group housing system (Grant and Albright, 2001), but it creates imbalance in individual energy intakes. Therefore, it is important to consider social factors when developing a feed management system.

1.4. Social stress

Farm animals are gregarious animals and highly motivated to form social groups. The benefits of an appropriate social environment for farm animals have been widely accepted, for instance enhanced feed intake, improved health and productivity (Albright and Arave, 1997). In natural conditions, group living reduces the risks of encountering predators whilst increasing available feeding time. When feed and other resources are insufficient, animals form smaller groups in order to avoid competition. For animals in captivity, feed accessibility and availability depends largely on management factors such as group size, frequency of feed delivery and design of the feed bunk. Animals cannot leave or join social groups according to the availability of resources. Therefore, in some cases, the costs of the social environment for captive animals could outweigh the benefits.

1.4.1. Social mixing

Compared to the lactation period, cows are more frequently regrouped during the late gestation to early lactation period (Cook and Nordlund, 2004). For example, at the end of lactation, cows may be grouped for drying-off, moved to a far-off dry group, followed by a close-up dry group, and then finally re-grouped as a calving group. It has been reported that regrouping of animals increases aggressive social interactions until the group stabilises (Cook and Nordlund, 2004). On commercial dairy farms, most agonistic social interactions can be seen in the feeding area (Val-Laillet et al., 2008b), suggesting that frequent regrouping would affect feed intake and feeding patterns of dry cows.

1.4.1.1. Effects of regrouping on feeding behaviour

The effect of regrouping on the feeding behaviour of dry cows has been reported by Schirmann et al. (2011). In this study, changes in the feeding behaviour of non-lactating cows (40±8d prior to expected calving) before and after regrouping were observed in an experimental trial, in which three cows were moved to a new pen and mixed with three resident cows. The feeding rate decreased after regrouping for both newly introduced and resident cows. The newly introduced cows decreased their DMI on the day of mixing, whilst the resident cows did not change their feed intake. Rumination activity of newly introduced cows also decreased for two days after mixing, but a decline in rumination was observed only on the day of mixing in the resident cows. These results indicate that regrouping of unfamiliar cows alters feeding behaviour, and that cows introduced to a new environment were more affected compared to cows that remained in their home pen.

However, this study also found that regrouping did not affect the frequency of feeder visits nor daily feeding time. A study on lactating cows also found no impact of regrouping on daily feeding time of one cow mixed into a group of eleven cows (von Keyserlingk et al., 2008). Feeding time of the focal cows in the hour after fresh feed delivery decreased on the day of mixing, but it returned to baseline (the average of the last three days before regrouping) on the day after regrouping. Both Schirmann et al. (2011) and von Keyserlingk et al. (2008) suggest that the effect of regrouping on feeding behaviour could be short-lived, diminishing within a few days after regrouping. Both studies observed regrouping of cows in a relatively small group size, and cows were regrouped only once. However, dry cows on modern commercial farms can be regrouped more frequently into large groups, suggesting that a frequent change in social structure may continuously disrupt the feeding behaviour of dry cows.

1.4.1.2. Effects of regrouping on feeding behaviour and energy metabolism

The effects of frequent regrouping during the prepartum period have been reported by Coonen et al. (2011). This study investigated the feeding behaviour, metabolic status and milk production in cows either kept in a socially stable group or a traditional dynamic social group during the close-up dry period. The former group had no cows added after the group was first established (n=10), whereas the latter group had a new cow joining the group of nine cows twice per week. There were no statistically significant differences in prepartum DMI and plasma nonesterified fatty acids (NEFA) concentrations, peripartum disease incidence and postpartum milk production between the stable and the traditional groups.

Studies on non-lactating Jersey cows in a larger group size (Silva et al., 2013a; 2013b; Lobeck-Luchterhand et al., 2014) also found similar results. These studies compared an all-in-all-out (AIAO) system with a traditional (TRD) system. The AIAO group had no new cows added to a starting group of 44 cows during five weeks prepartum. The TRD group had a median of 9.0 (Silva et al., 2013a; 2013b) or 10.4 (Lobeck-Luchterhand et al., 2014) new cows added to the group every week to maintain a group size of 44. There were no statistically significant differences between AIAO and TRD groups in peripartum disease incidence, culling rate, lameness score, milk production, milk fat and protein content, body condition score, or reproductive parameters (Silva et al., 2013b).

Lobeck-Luchterhand et al. (2014) found that the feeding time of AIAO cows was shorter in the first week compared to TRD cows, but this trend was reversed in the second week, with no significant differences from the third week onwards. Regardless of grouping strategy, most cows were seen at the feed bunk after fresh feed delivery, and there was no difference in weekly feeding patterns between AIAO and TRD cows (Lobeck-Luchterhand et al., 2014).

The authors suggested that sufficient feed bunk space for both groups (92% of feed bunk stocking density) may have eliminated the effects of the grouping strategy on feeding patterns. Moreover, Silva et al. (2013a, 2013b) found no effect of grouping strategy on serum cortisol concentrations, plasma β-hydroxybutyric acid (BHB) and NEFA concentrations, innate immune parameters and immunoglobulin (Ig) G concentrations in the colostrum. It appears that frequent regrouping of prepartum dairy cows may have negligible negative effects on feeding behaviour, health and milk production, provided that there is sufficient feeding space.

1.4.1.3. Effects of regrouping on agonistic social interactions at the feeder

Although frequent regrouping did not affect the feeding behaviour of cows, more frequent agonistic interactions were observed in the TRD group after feed delivery (Lobeck-Luchterhand et al., 2014). In this study, agonistic interactions were defined as the frequency of displacement, and a displacement rate (the number of displacements as a proportion of group size) was calculated to take into account the change in group size in the AIAO group. The frequency of displacements and the displacement rate were significantly lower in the AIAO group compared to the TRD group in week one, three and five. The difference in the displacement rates was most pronounced in week one (0.78±0.07 displacements/day for TRD, 0.33±0.06 displacements/day for AIAO). In both groups, displacements were more frequently observed in week one than in week five. In contrast, in a stable pen management proposed by Coonen et al. (2011), it was reported that a single cow entering a group of ten cows did not affect the frequency of displacements in the hour after fresh feed delivery.

Both Lobeck-Luchterhand et al. (2014) and Coonen et al. (2011) presented agonistic social interactions as a group average. However, Mench et al. (1990) pointed out that newly introduced animals were more severely affected by social mixing, compared to those that remained in their home environment. An earlier study (Brakel and Leis, 1976) reported that newly introduced cows were involved in agonistic social behaviours more frequently than resident cows after mixing. More recent studies (von Keyserlingk et al., 2008; Schirmann et al., 2011) also found similar results, where peak feeding time observations showed that the number of competitive interactions initiated by resident cows was consistent before and after regrouping, and only newly introduced cows increased the frequency of agonistic social interactions. For example, cows moved to a new pen gradually increased the number of displacements initiated (actor) during the three hours after feed delivery on the day of mixing compared to the day before mixing (Schirmann et al., 2011). However, the number of displacements received (reactor) and the number of replacements (actor took over the feeder) were not different before and after the mixing.

In contrast, results in lactating cows (von Keyserlingk et al., 2008) indicated that there was no change in the frequencies of displacements initiated at the feed bunk when cows were moved to a new pen, but the number of displacements received increased after regrouping. The reason for this discrepancy between the two studies is uncertain, but may be due to different grouping strategies and/or different feed bunk designs (feed bins: Schirmann et al., 2011; post-and-rail feed barrier: von Keyserlingk et al., 2008).

Von Keyserlingk et al. (2008) investigated social interactions at lying stalls after regrouping. Again, they found that behavioural changes after regrouping were only observed in cows that were introduced to a new group. They found that the newly introduced cows were less likely to be involved in competition for the stall after regrouping, compared to the days before mixing (2.0±0.4 times/d and 0.4±0.4 times/d, before and after mixing, respectively). The changes in the agonistic social interactions at lying stalls of newly introduced cows were only observed for one day after mixing, and there was no change in the number of displacements for resident cows. Additionally, lower frequency bouts of allo-grooming were initiated by newly introduced cows during the three days after mixing, and these cows received fewer grooming bouts from the resident cows on the day of mixing.

Social mixing does not only affect the social behaviour of cows. A modest increase in the frequencies of lying bouts after mixing was observed in the newly introduced cows, which may indicate “some degree of restlessness” (Schirmann et al., 2011). von Keyserlingk et al. (2008) found a significant decrease in lying bouts and lying time in the newly introduced cows on the day of mixing. However, changes in lying bouts and lying time were observed only on the day of mixing, and both lying bouts and lying time returned to baseline levels on the following day.

1.4.1.4. Mixing primiparous cows with multiparous cows

It has been reported that cows with lower social rank are most affected by regrouping (Collings et al., 2011; DeVries et al., 2004; Olofsson, 1999). Primiparous cows are normally lower in social rank when housed in groups with adult cows (González et al., 2003), suggesting that first calving cows are likely to suffer from adverse effects of regrouping when they enter the lactating herd for the first time. Phillips and Rind (2001) reported that primiparous cows were involved in more competition for feed and more allo-grooming, and spent a greater time standing if they were grouped with multiparous cows, compared to primiparous cows introduced to a group consisting of primiparous cows only. Unfavourable effects of mixing primiparous cows with adult cows can be attenuated by mixing heifers with adult cows before their first lactation. Compared with heifers mixed after calving, heifers mixed with multiparous cows before calving were involved in fewer agonistic interactions after joining the lactating group (Boyle et al., 2013; de Vries et al., 2015). However, these studies did not investigate any reactions of heifers when they were mixed with the dry cows, but it is likely that those heifers were involved in agonistic social interactions with the dry cows at mixing. Nevertheless, a change in social environment before calving may be less stressful compared to the levels of stress they would experience upon entering the lactating herd.

1.4.1.5. Summary of social mixing in cows

The effect of mixing on the behaviour of cows appears to be relatively mild and short-lived. The change in the number of agonistic social interactions at the feed bunk and at lying stalls returned to baseline within three days after mixing (von Keyserlingk et al., 2008), and the weekly regrouping event appeared to have no significant impact on metabolism, physiology and production (Silva et al., 2013a; 2013b). Bøe and Færevik (2003) pointed out that the overall level of aggression in cattle is lower than in pigs. Cows are highly motivated to socialise, and are easily accustomed to regrouping when it was repeated (Kondo et al., 1984; Veissier et al., 2001). Phillips and Rind (2001) reported that the occurrence of agonistic social interactions was affected by group size, group composition, timing of mixing and feed availability. Additionally, it has been reported that agonistic social interactions at regrouping can be decreased by reduced stocking density (Talebi et al., 2014). This would suggest that stocking density can have a bigger impact on the feeding and social behaviour of cows compared to regrouping.

1.4.2. Stocking density

Overstocking is a common feature of large commercial dairy farms. This practice is used to maximise profit per unit whilst reducing cost. However, the negative impacts of intensive housing systems on the welfare and productivity of farm animals have been pointed out by Petherick and Phillips (2009). In dairy cows, a number of studies have investigated the effects of high stocking density or limited space allowance on the performance of both lactating and dry cows. The experimental design of these studies can be divided into four types (**Table 1.1**): studies that investigated the effects of: 1) overstocking of the feed-face or restricted feed-face space; 2) overstocking of both the feed-face and the lying area; 3) overstocking of the lying area; 4) understocking of both the feed-face and the lying area.

1.4.2.1. Effect of high stocking density on agonistic social interaction at the feeder

Regardless of the experimental setting, increased agonistic social interactions were observed due to limited feed-face space (DeVries et al., 2004) or increased stocking density at the feed-face (Olofsson, 1999; DeVries et al., 2004; Huzzey et al., 2006, 2012; Fregonesi et al., 2007a; Proudfoot et al., 2009; Collings et al., 2011; Krawczel et al., 2012a; Lobeck-Luchterhand et al., 2015). However, overstocking of the lying area did not increase agonistic interactions (Telezhenko et al., 2012). It has been reported that agonistic social interactions in cows are mainly seen in the feeding area (Val-Laillet et al., 2008b) and are most frequently observed during the peak feeding period (e.g. after fresh feed is delivered: DeVries et al., 2004; Lobeck-Luchterhand et al., 2014; Proudfoot et al., 2009; within 0.5h after milking: Olofsson, 1999). This suggests that increased competition due to high stocking density and limited feeding space could disrupt the feeding behaviour of cows.

1.4.2.2. Effect of competition at the feed-face on the feeding behaviour of cows

Hill et al. (2009) and Krawczel et al. (2012a) reported that four different stocking densities at both the feed-face and the lying area (100%, 113%, 131% and 142%) did not affect the daily feeding time of lactating cows. However, cows had longer daily feeding times at 100% feed-face stocking density compared to 142% (Krawczel et al., 2012b), or when the feeding space was increased from 0.33 yoke/0.21m feed-face space per cow to 1.33 yoke/0.81m feed-face space per cow (Huzzey et al., 2006). DeVries et al. (2004) also found that cows increased feeding time by 10% and feeding activity by 14% when feed-face space was increased from 0.5 m per cow to 1.0 m per cow. The greater feeding time at low stocking density or with wider feed-face space availability was most pronounced during the peak feeding period (DeVries et al., 2004; Huzzey et al., 2006, 2012; Collings et al., 2011). Huzzey et al. (2012) also reported that cows took longer to approach the feeding area after fresh feed delivery when overstocked. These results indicate that a reduced stocking density or a greater space allowance enabled more cows to access feed, and allowed cows not to have to compete for feed.

Olofsson (1999) reported that cows in an overstocked situation (four cows/feeder) had a higher eating rate and visited the feeder more frequently compared to cows in a control group (one cow/feeder), resulting in a significant increase in DMI. A higher feeding rate was also observed in a less crowded situation (two cows/feeder: Collings et al., 2011; Hosseinkhani et al., 2008; Proudfoot et al., 2009). These studies also reported a decrease in DMI during the peak feeding period (Hosseinkhani et al., 2008; Collings et al., 2011), or in multiparous cows (Proudfoot et al., 2009). However, the effects of competition at the feed-face on daily DMI were not consistent between studies, reporting either an increase (Huzzey et al., 2012) or no change in daily DMI due to overstocking (Collings et al., 2011; Krawczel et al., 2012b). This discrepancy may be because of a shorter treatment period (one week) in Collings et al. (2011) compared to 14 days (Huzzey et al., 2012), or a different experimental setting (**Table 1.1**).

Competition at the feed-face can affect other feeding activities. For example, Hosseinkhani et al. (2008) found that cows at high stocking densities had fewer meals per day, a higher feeding rate, but spent longer time feeding, and consumed a larger amount in each meal. Although this study did not find any associations between competition at the feed-face and feed sorting behaviour, the authors suggested that the changes in feeding behaviour seen in competitive situations can create larger individual variations in consumption of the TMR. Higher stocking densities in the lying area also affected rumination activity. When access to cubicles was restricted to 0.76 cubicles per cow or less (Krawczel et al., 2012a; 2012b), cows spent less time standing/lying on a cubicle while ruminating compared to when each cow had access to 1.0 cubicle. Overall daily rumination (at a cubicle or other locations) was not affected by stocking density (Krawczel et al., 2012a; 2012b; Fustini et al., 2017). It is unknown whether posture or location during rumination affects digestibility of feed and metabolism, but this would be worth further investigation.

Table 1.1. Summary of experimental design (H: high, L: low) and main results of the effect of stocking density in dairy cows.

	Treatment period	Feed-face	Lying area	Main findingsat high stocking density
DeVries et al. (2004)	Mid lactation (9 days)	H) 0.5m/cow L) 1.0m/cow	1.0 cubicle/cow	displacements↑, feeding time↓ inter-cow distance↓
Huzzey et al. (2006)	Mid lactation (10 days)	Yoke barrier: 1.33/1.00/0.67/0.33 yoke/cow Post-and-rail barrier: 0.81/0.61/0.41/0.21m/cow	1.0 cubicle/cow	displacement↑ (yoke<post&rail) feeding time↓ (yoke<post&rail) standing inactive↑
Hosseinkhani et al. (2008)	Dry (23 days)	H) 0.5 feed bin/cow L) 1.0 feed bin/cow	Not Studied	feeding rate↑, peak time intake↓
Fregonesi et al. (2007a)	Mid lactation (1 week)	Not Studied	12, 11, 10, 9, 8 cubicles/12 cows (100, 109, 120, 133, 150%)	displacements (lying area)↑ lying time↓
Proudfoot et al. (2009)	Dry (18 days)	H) 0.5 feed bin/cow L) 1.0 feed bin/cow	Not Studied	displacements↑ (primi:×3, multi:×2) multi: standing without eating↑
Olofsson (1999)	Mid lactation (1 week)	H) 0.25 feed bin/cow L) 1.00 feed bun/cow	Not Studied	displacements↑, feeding rate↑, intake↑
Huzzey et al. (2012)	Dry (14 days)	H) 0.34m/cow L) 0.67m/cow	H) 0.5 cubicle/cow L) 1.0 cubicle/cow	displacements↑, peak time feeding↓ energy metabolism↓ faecal cortisol metabolites↑(primi)
Lobeck-Luchterhand et al. (2015), Silva et al. (2016, 2014)	Dry (28 days)	H) 1.0 yoke/cow (100%) L) 1.3 yoke/cow (80%)	H) 1.0 cubicle/cow (100%) L) 1.3 cubicles/cow (80%)	displacements↑, disease incidence- lying time↓, serum/hair cortisol-
Fustini et al. (2017)	Dry (21 days)	H) 0.66m/cow L) 1.20m/cow	H) 3.3m^2 lying area/cow L) 7.8m^2 lying area/cow	number of steps/h↑, plasma cortisol↑ lying time-, ruminating activity-
Hill et al. (2009), Krawczel et al. (2012a, 2008)	Mid lactation (7 days)	1.00, 0.88, 0.76, 0.71 yoke/cow (100, 113, 131, 142%)	1.00, 0.88, 0.76, 0.71 cubicle/cow (100, 113, 131, 142%)	displacements↑, standing time↑, faecal cortisol metabolites-
Krawczel et al. (2012b)	Mid lactation (14 days)	H) 0.71 yoke/cow (142%) L) 1.00 yoke/cow (100%)	H) 0.71 cubicles/cow (142%) L) 1.00 cubicle/cow (100%)	feeding time↓, lying time↓, ruminating activity in stall↓

↑: significant increase, ↓: significant decrease, -: no difference

1.4.2.3. Effect of high stocking density on the health and metabolism of prepartum cows

The changes in feeding behaviour seen as a result of high stocking densities would suggest that the energy status of cows could also be affected. Huzzey et al. (2012) investigated the effect of different stocking densities on the metabolism of prepartum cows by measuring concentrations of plasma NEFA, insulin and glucose. Higher concentrations of plasma NEFA and glucose and a slower glucose clearance from circulation were observed when cows were overstocked (0.34m feed-face space per cow, 0.5 cubicles per cow), suggesting altered energy metabolism. The authors noted that these results were similar to the physiological responses of cows to poor nutritional supply, despite higher DMI during the overstocked period. This study indicated that overstocking could compromise energy metabolism in prepartum dry cows, potentially increasing the risk of peripartum diseases as discussed in the previous section.

Another study on prepartum Jersey cows (Silva et al., 2014) found no effect of stocking density on peripartum metabolic parameters (plasma NEFA and BHB) when comparing 100% stocking density (1.0 yoke and 1.0 cubicle per cow) with 80% stocking density (1.3 yokes and cubicles per cow). This study also did not find any differences between stocking densities in peripartum disease incidence and changes in body condition score. The authors argued that behavioural alterations due to high stocking density may not always be associated with changes in health and metabolic parameters. Ensuring that cows have at least 1.0 yoke and 1.0 cubicle may have resulted in no detrimental effect on the metabolic status of prepartum cows.

1.4.2.4. Effects of high stocking density on stress physiology

Huzzey et al. (2012) measured faecal cortisol metabolite levels (11,17-dioxoandrostane: 11,17-DOA) to investigate cortisol secretion in responses to overstocking. They found that the concentration of 11,17-DOA in faeces was significantly higher during a period of overstocking in primiparous cows, suggesting that primiparous cows may have experienced stress due to increased agonistic interactions. This study also investigated adrenal activity by measuring plasma cortisol in response to ACTH challenge but found no association between stocking density and adrenal activity. Fustini et al. (2017) measured the level of plasma cortisol and dehydroepiandrosterone (DHEA) as indicators of acute stress, and found elevated levels of prepartum plasma cortisol and DHEA in overstocked cows (0.66m feed-face space and $3.3m^2$ resting area per cow) compared to cows in the control group

(1.20m feed-face space and 7.8m^2 resting area per cow).

In contrast, a study on lactating cows (Krawczel et al., 2012a) reported that there were no differences in the level of faecal glucocorticoid metabolites (11-oxoetiocholanolone) between stocking densities of 100%, 113%, 131% and 142% at both the feed-face and the lying area. This discrepancy could be due to the different level of high stocking densities at the feed-face and the lying area between two studies. It has been reported that cows prioritise lying time over feeding if access to both resources is restricted (Metz, 1985; Munksgaard et al., 2005). Huzzey et al. (2012) did not observe lying behaviour, but Krawczel et al. (2012a) found a decrease in daily lying time under high stocking conditions.

Associations between reduced daily lying time and an increased physiological stress response have been reported in dairy cows, especially when access was restricted to 0.5 cubicles per cow or less (Friend et al., 1979; González et al., 2003). Therefore, Krawczel et al. (2012a) argued that their experimental setting and an associated deprivation of lying may not have been severe enough to induce physiological responses to overstocking. Similarly, a study on prepartum Jersey cows (Silva et al., 2016) found that prepartum serum cortisol concentrations and hair cortisol concentrations at calving were unaffected by stocking density when all cows were given access to at least 1.0 yoke and cubicle per cow. Therefore, these results suggest that a severe deprivation of lying behaviour due to overstocking of the lying area may be more stressful to cows compared to overstocking of the feeding area, and may have triggered a physiological stress response.

1.4.2.5. Effect of stocking density on lying/standing behaviour

Telezhenko et al. (2012) reported that cows reduced their time spent lying when the availability of cubicles was decreased from four cubicles per cow (25% stocking density) to one cubicle per cow (100% stocking density). However, the number of lying bouts and bout durations of prepartum Jersey cows (Silva et al., 2014) were unchanged by a decreased availability of cubicles (stocking density) from 1.3 cubicles per cow (80%) to one cubicle per cow (100%). However, when the availability of cubicles was reduced to less than one cubicle per cow (i.e. >100% stocking density: Fregonesi et al., 2007a; Hill et al., 2009; Krawczel et al., 2012a, 2012b), cows decreased their daily lying time compared to 100% stocking density. Fregonesi et al. (2007a) also observed more frequent competition for cubicles when the stocking density exceeded 100%. In contrast, it appears that daily lying time was unaffected when only the feed-face was overstocked, and all cows had access to at least one cubicle (Collings et al., 2011; Olofsson, 1999). Instead, overstocking of the feed-face led to cows increasing the time spent standing inactive (without eating) in the feeding area, regardless of the stocking density of the lying area (Olofsson, 1999; Huzzey et al., 2006; Krawczel et al., 2008; Hill et al., 2009; Proudfoot et al., 2009).

Fustini et al. (2017) reported that daily lying time and lying bout frequency was not changed when the resting area decreased from 7.8m^2 to 3.3m^2 per cow, although the latter treatment increased the number of steps per hour. Similarly, Fregonesi and Leaver (2002) reported that there was no difference in daily lying time and lying time (min/h) during the peak lying periods (01:00-05:00h, 10:00-13:00h) when the space allowance of the straw yard was reduced from 9.0m^2 to 4.5m^2 per cow. The same study also found that when space allowance for cubicle housing was decreased from 1.50 cubicles to 0.88 cubicles per cow, daily lying patterns were disrupted, and lying time (m/h) during the peak lying periods was reduced. This suggests that overstocking of cubicle housing has a greater impact on the lying behaviour of cows compared to straw yards, which explains the lack of observed difference in lying behaviour in the study by Fustini et al. (2017). In straw yards, cows can adjust their distance from neighbouring cows when the space allowance is limited, which allows for all cows to lie down at the same time. In contrast, lower availability of cubicles leads to some cows (most likely subordinate cows) being displaced by dominant cows and standing for longer durations.

1.4.2.6. Other factors affecting competition at the feed-face

Collings et al. (2011) found that competition at the feed-face was greatest when overstocked at 200% (0.5 feeders per cow), and where the availability of feed was restricted to 14 hours per day. This treatment (high stocking and restricted feed availability) also decreased feeding activity during the peak feeding period. Competition at the feed-face was not affected by feed availability (*ad libitum* or restricted) at 100% stocking density (one feeder per cow), but *ad libitum* feed availability decreased the frequency of displacements at the feed face at 200% stocking density. Temporal feed restriction resulted in reduced total daily DMI, daily feeding time and total number of feeder visits, irrespective of the stocking density. Feeding activity during the peak feeding period was reduced when feed was always available compared to temporal feed restriction. This suggests that *ad libitum* provision of feed allowed cows to distribute their feeding activity throughout the day, whilst cows with restricted feed delivery concentrated their intake at peak feeding time (i.e. immediately after fresh feed delivery). The authors concluded that stocking density at the feed-face was a more important factor in the increase in competition at the feed-bunk compared to feed availability, highlighting the importance of adequate feed bunk space. However, this study also suggested that feed availability is also an important factor affecting the feeding pattern of cows.

It has been reported that the type of feed barrier system (yoke or post-and-rail system) affects the number of displacements at the feed-face and feeding activity during the peak feeding period (Endres et al., 2005). There was less competition at the feed-face when the yoke feed barrier system was used, but with the post-and-rail system more cows were observed in the feeding area and cows spent more time feeding during the peak feeding period. The yoke system may help reduce competition at the feed-face whilst facilitating more equal access to feed for subordinate cows (Endres et al., 2005).

To investigate how overstocking of these different feed barrier systems affected competitive behaviour of cows, Huzzey et al. (2006) created four different stocking densities for both the yoke system (1.33, 1.00, 0.67, 0.33 yoke per cow) and the post-and-rail system (0.81, 0.61, 0.41, 0.21m of linear bunk space per cow). Regardless of the feed barrier system, the average number of displacements increased and daily feeding time decreased as the stocking density increased. However, the levels of increased competition and decreased feeding time were greater in the post-and-rail system compared to the yoke system. Moreover, an index of success for individual cows was calculated in this study (index of success=number of cows that an individual is able to displace/(number of animals that an individual is able to displace + number of animals that are able to displace the individual)×100: Mendl et al., 1992). They found that those cows with a lower index of success were less likely to be displaced at the yoke system compared to the post-and-rail system. Therefore, the yoke barrier system can reduce agonistic social interactions at high stocking densities and protect subordinate cows from being displaced.

1.4.2.7. Social status and parity

Social rank played an important role in determining feeding activity with restricted space availability (Friend et al., 1977). DeVries et al. (2004) found that the feeding activity of dominant cows during the peak feeding period was not affected by feed-face space availability. However, subordinate cows that had the lowest peak time feeding activity with limited feed-face space (0.5m per cow) increased their feeding activity by 24% when wider feed-face space (1.0m per cow) was provided. Similarly, Olofsson (1999) found that subordinate cows in an overstocked situation changed their feeding time, eating longer at night to avoid competition. Collings et al. (2011) reported that when feed was not available at night, competition was more intense after the morning feed delivery, and subordinate cows had a difficult time competing. These results suggest that cows in lower social ranks would benefit from better feed bunk management e.g. increased feed bank space, *ad libitum* feed availability and frequent feed delivery.

Proudfoot et al. (2009) reported a parity difference in reactions to overstocking. An increase in displacements at high stocking density was more significant in primiparous cows compared to multiparous cows (three times and twice as much competition compared to non-competitive groups, respectively). However, changes in feeding behaviour due to higher stocking densities were mainly observed in multiparous cows, and not in primiparous cows. For example, multiparous cows had a faster feeding rate, especially for those frequently displaced from an individual feeding station. Multiparous cows in an overstocked condition spent a longer time standing without eating at the feeder at the high stocking density. This resulted in a decrease in meal duration (feeding time per visit) by 28% compared to a non-competitive situation.

Huzzey et al. (2012) also found that primiparous cows took longer to approach the feeding area when overstocked, but this was not observed in multiparous cows. Moreover, this study found a difference in physiological responses to overstocking between primiparous and multiparous cows. Overstocking of the feed-face and the lying area resulted in increased levels of plasma NEFA, glucose and faecal 11,17-DOA in primiparous cows, but multiparous cows only showed elevated plasma NEFA levels. These two studies suggest that physiological responses to overstocking were more severe in primiparous cows compared to multiparous cows, and that multiparous cows were probably better at coping with social pressure due to high stocking density, by avoiding competition at the feed-face and changing their feeding behaviour.

1.4.3. Social stress in dry cows

From the studies described above, it is evident that the social environment of dairy cows can have significant impacts on their feeding and lying behaviours. The effect of social mixing may be more pronounced in primiparous cows than multiparous cows. In the studies described above, cows experienced a high stocking density between one and four weeks during the prepartum period. On modern farms, however, cows may be overstocked for a much longer period, and continuous overstocking and/or frequent regrouping may have a more severe effect. In dairy production systems where changes in social structure can be most dynamic between dry-off and calving (Cook and Nordlund, 2004), cows in the dry period could be subjected to greater changes in social environment, which may affect performance during the subsequent lactation period. Further studies with more industry-relevant experimental settings are warranted to investigate the long-term effects of social stress on prepartum cows.

1.5. Environmental factors

1.5.1. Thermal stress

1.5.1.1. Heat stress in dairy cows

The health, productivity and welfare of livestock can be influenced by environmental factors which include air temperature, humidity, and durations of light exposure (Dahl et al., 2000; Kadzere et al., 2002; Collier et al., 2006; Tao and Dahl, 2013). A cow's body temperature is maintained when it is between the lower and upper critical temperatures (Kadzere et al., 2002). The lower and upper critical temperatures reported in lactating cows are -30°C (during peak lactation: Young, 1983) and 25-26°C (Berman et al., 1985), respectively. When the air temperature reaches above 26°C, cows lose homeothermy and can enter heat stress (Kadzere et al., 2002). Humidity also affects animal comfort; hence the temperature-humidity index (THI) is often used to evaluate levels of thermal stress (Bohmanova et al., 2007).

Various studies in dairy cows have reported that high temperature and/or THI above threshold (usually THI of 72, established by Armstrong, 1994) were associated with compromised performance. For example, a reduction in DMI and milk production (West et al., 2003), poor reproductive performance (Moore et al., 1992; Avendaño-Reyes et al., 2006) and increased mortality (Vitali et al., 2009; Crescio et al., 2010) have been reported in dairy cows experiencing heat stress. All of this would result in lower profitability (St-Pierre et al., 2003). Observational studies also showed that cows experiencing hot seasons during the late gestation period (i.e. dry period) produced less milk during the subsequent lactation (Moore et al., 1992; Thompson and Dahl, 2012; Tao and Dahl, 2013). Moreover, records from 2,613 dairy cows on commercial farms in the United States (US) over three years showed that cows experiencing their dry period in summer months had the highest incidence of metabolic and infectious diseases in the first 60 days after calving (Tao et al., 2012b).

1.5.1.2. Effect of cooling of dry cows

There are controlled studies on dry cows that have investigated the effects of different methods of cooling during the late gestation period on cow performance. These methods included provision of shade (Collier et al., 1982), wetting and forced ventilation (Wolfenson et al., 1988), and the use of fans and sprinklers (Adin et al., 2009; do Amaral et al., 2009, 2011, Tao et al., 2011, 2012b). All of these methods successfully reduced rectal temperatures and/or respiration rates of dry cows and improved milk production during the subsequent lactating period. Improved prepartum dry matter intake and body weight gain have also been reported (Adin et al., 2009; Tao et al., 2011, 2012b).

1.5.1.3. Metabolic and immune responses to heat stress

It has been reported that lactating cows under heat stress were more likely to be in negative energy balance compared to lactating cows in a controlled temperature (Wheelock et al., 2010). Similarly, pregnant heifers under heat stress had a compromised metabolic status shown by lower plasma glucose levels and higher plasma NEFA and BHB levels compared to heifers in a thermoneutral condition (Nardone et al., 1997). In contrast, studies on dry cows (Collier et al., 1982; do Amaral et al., 2009; Tao et al., 2012b) found no differences in metabolic profiles (plasma concentrations of glucose and NEFA) between cows under heat stress and cows with a cooling treatment. Tao and Dahl (2013) argued that the difference in metabolic responses to heat stress between lactating and dry cows may be due to the different energy status of cows at these different stages of the production cycle. Kadzere et al. (2002) also suggested that dry cows were less sensitive to high temperature as they produce less heat compared to lactating cows.

The effect of heat stress on immune function has been reported by do Amaral et al. (2011). Dry cows under heat stress had an impaired acquired immune function (as measured by immunoglobulin levels after ovalbumin challenge), compared to dry cows cooled with fans and sprinklers. They also found an impaired postpartum neutrophil function (2-20 days after calving) in heat-stressed cows, indicated by a greater oxidative burst and phagocytosis. Additionally, poorer cell mediated immune function (reduced proliferation of peripheral blood mononuclear cells) was found in cows under heat stress compared to cows under a cooling treatment (do Amaral et al., 2010). Furthermore, Adin et al. (2009) reported that colostrum quality and quantity were reduced by heat stress during the dry period, but could be improved by cooling cows using evaporative cooling in both tie stall and free stall housing systems. However, Tao et al. (2012a) found no significant difference in colostrum Ig level between cows under heat stress and cows cooled with fans and sprinklers during the dry period. This may be due to a different length of cooling period used in the two studies (eight weeks: Adin et al., 2009; 46 days: Tao et al., 2012a).

1.5.1.4. Physiological stress responses to heat stress

A study conducted in Italy (Lacetera et al., 2005) found that cows that gave birth in summer had higher prepartum concentrations of plasma cortisol compared to cows that gave birth in spring. Spring calving cows never experienced an environment above the upper critical THI for dairy cows, while the THI for summer calving cows went above this threshold during the day. In contrast, Tao et al. (2012a) did not find any difference in circulating cortisol levels during the dry period and at calving between heat stressed cows and cows that were cooled with fans and sprinklers. These two studies indicate that the stress levels of prepartum cows increase when the THI exceeds the critical limit for cows, but cooling with fans and sprinklers may not always attenuate the physiological responses to heat stress.

1.5.1.5. Lying/standing behaviour of cows under heat stress

More recent studies have reported that the standing behaviour of cows is altered by heat stress (Karimi et al., 2015) and increased core body temperature (Allen et al., 2015). Allen et al. (2015) reported that the standing behaviour was affected when the THI reached 68 or core body temperature exceeded 38.8°C. Cows were more likely to stand up and remain standing when they had higher core body temperatures. Cows with a high core body temperature extended their standing bout duration, as cows were less likely to lie down. Similarly, Karimi et al. (2015) reported that prepartum cows under heat stress had longer daily standing times (7.9h/d) compared to prepartum cows cooled with fans and sprinklers (6.5h/d). This study also reported that heat stress decreased daily rumination and chewing times compared to cows receiving cooling treatment. Together with the decreased feed intake of heat stressed cows, extended standing times and decreased rumination can negatively affect the health and welfare of dairy cows. Although non-lactating cows produce less metabolic heat than lactating cows (West, 2003), it is evident from the studies described above that dry cows can suffer in hot weather. Cooling dry cows can effectively reduce the negative impact of heat stress, but probably does not completely eliminate stress for cows.

1.5.1.6. Cold stress in dairy cows

Compared to heat stress, literature on cold stress in dairy cows is limited, probably because dairy cows rarely experience conditions where the air temperature is below their lower critical temperature (LCT for indoor cows is -25°C according to NRC, 1981). However, in a cold environment, cows need to increase their metabolic rate and feed intake to maintain homeothermy, while reducing production levels (Young, 1981, 1983; Brouček et al., 1991; Zähner et al., 2004). Therefore, cows may experience stress due to cold weather even before reaching LCT. In places where winter weather is relatively mild, it is common to keep cows on pasture in winter (New Zealand: Tucker et al., 2007; Webster et al., 2008; Ireland: Boyle et al., 2008; O'Driscoll et al., 2010). Although pasture-based dairy farming has benefits such as lower management costs and better welfare, the exposure to harsh winter weather may not always be beneficial for cows.

It has been reported that out-wintering of prepartum cows can promote natural behaviour (e.g. synchronised lying) without compromising subsequent milk production. This may suggest that this system can be a suitable alternative to a confinement system (O'Driscoll et al., 2008, 2010). Similarly, Boyle et al. (2008) reported that out-wintering pregnant heifers enhanced grooming, social and play behaviour and reduced limb injuries compared to heifers kept indoors. However, DMI for out-wintered heifers was lower than indoor heifers, and out-wintered heifers gained less weight and less body condition during the out-wintering period compared to indoor heifers. The authors argued that lower DMI in out-wintered heifers may be due to lower palatability and lower quality of their diet which were affected by the weather, and stressed that the benefits of an out-wintering pad system are largely influenced by climatic and management conditions.

Tucker et al. (2007) and Webster et al. (2008) artificially created extreme cold conditions using fans and sprinklers to assess the behavioural and physiological responses of non-lactating cows to a harsh winter environment. Both studies found that cows spent less time eating and lying when they were kept outdoors compared to when they were kept indoors. Tucker et al. (2007) reported that cows changed their lying posture in such a way that they minimised the exposure of their body to wind and rain, and that cows were more likely to stand with their head down or stand next to windbreaks (e.g. feed troughs). Both studies also found higher concentrations of plasma cortisol and faecal glucocorticoid metabolites in cows kept outside, suggesting that the wet and windy conditions induced a physiological stress response. Cows kept outside also had increased concentrations of NEFA, suggesting negative energy balance and enhanced fat mobilisation. Moreover, Tucker et al. (2007) reported that cows with lower body condition scores were most affected by the cold weather, which was evident in changes in lying and standing postures and lower body fat mobilisation. This highlights the importance of maintaining adequate body condition of cows subjected to cold conditions.

Redbo et al. (2001) found that heifers decreased feeding activity in cold weather, and increased their lying time when the outside temperature and sun radiation were low. Similarly, a negative correlation between lying duration and THI has been reported in dairy cows (Zähner et al., 2004). Indeed, large wild ungulates spend 40-50% or more time lying in winter (caribou: Adamczewski et al., 1993; reindeer: Cuyler and Øritsland, 1993). The contradictory results in lying time between the studies of Redbo et al. (2001), Tucker et al. (2007) and Webster et al. (2008) may be due to the condition of the lying area. It has been reported that cows spent less time lying when the surface of the lying area is wet (Fregonesi et al., 2007b). The extended standing time of cows found by Tucker et al. (2007) and Webster et al. (2008) may be due to a wet lying area surface, whereas the lying area in Redbo et al. (2001) was kept dry. Increased standing behaviour due to wet conditions was also observed in out-wintered beef cows (Morgan et al., 2009).

Tucker et al. (2007) and Webster et al. (2008) suggested that the activation of the stress axis observed in cows from their studies might in part be caused by an extended standing time due to the wet lying area surface, rather than the cold temperature. Indeed, Zähner et al. (2004) did not find any association between THI and concentrations of cortisol in milk. Cows in the study by Tucker et al. (2007) had an extremely short lying time (4h/24h), and such reductions in lying time are associated with poor welfare (Jensen et al., 2005; Munksgaard et al., 2005)

When experiencing high wind speed, out-wintered dairy heifers preferred to spend their lying time in the dry lying area or in the forest, rather than in open fields (Redbo et al., 2001). This study also found that heifers spent less time at the feed bunk when it was sited in open fields in harsh cold weather conditions (low temperature, low solar radiation, strong wind). On the other hand, when a windbreak was not provided (Tucker et al., 2007), cows were more often observed standing near feed troughs with their head down. This is in agreement with the findings from a study on out-wintered beef cattle (Morgan et al., 2009), where beef cattle spent more time near the feed bunk, trees or shelters when the wind speed was high.

Although out-wintering during the dry period can sustain subsequent milk production (O'Driscoll et al., 2010), there are changes in behaviour that occur as a result of the low temperature, wind and rain. Cows seek out windbreaks and adapt their behaviour to minimise heat loss and energy expenditure. Deprivation of lying behaviour due to a wet lying area could be stressful to cows, and feed intake might be affected by compromised palatability and quality of feed in the outside feeding area. These findings suggest that it is likely that the behaviour of out-wintered cows is influenced by management conditions, as well as the cold and harsh weather conditions. It is essential to provide shelters such as windbreaks, and keep the lying and feeding areas dry.

1.5.2. Housing systems

Housing systems, bedding and floor surfaces can be important factors in cow welfare, as they affect leg and hoof health (Cook and Nordlund, 2009). Haskell et al. (2006) reported that farms that allow grazing for cows had a lower incidence of lameness compared to farms implementing a zero-grazing system. Associations between higher daily grazing hours and lower risks of hock injury have also been reported (Burow et al., 2013). Hernandez-Mendo et al. (2007) reported that the gait scores of lame cows can be improved by grazing for four weeks. Therefore, it appears that grazing during the dry period could provide better welfare for dry cows, by improving their leg and hoof health. However, grazing is not always a feasible option for farms with limited grazing land or under extreme weather conditions.

A continuous housing system is more commonly used by farms with high yielding herds due to its convenience for feeding management (Haskell et al., 2006). In an indoor system, bedding quality is an important determinant of hoof and leg health. It has been reported that sand bedding (Cook et al., 2004) or dry sawdust bedding (Fregonesi et al., 2007b) on cubicles were associated with increased time spent lying on cubicles. Hard floor surfaces are associated with a greater risk of hoof damage and lameness (Somers et al., 2003).

According to a survey conducted on organic and non-organic farms in the UK (Langford et al., 2009), passageways and lying areas for dry cows were generally not clean in either of the farms. An exposure to manure on the floor would also increase the risk of digital dermatitis (Somers et al., 2005). Moreover, Green et al. (2007) reported that poor hygiene levels of dry cows in both cubicle sheds and straw yards were associated with risks for mastitis after parturition. Fregonesi and Leaver (2001) found that cows kept in straw yards were dirtier than cows kept in cubicle housing. These studies highlight the importance of hygiene and soft floor surfaces of the housing system for udder and leg health.

Barker et al. (2007) found that cows kept in a straw yard during the dry period had higher locomotion scores when entering the lactation period, compared to cows kept in cubicle housing during the dry period. The authors argued that the change from the soft surface of the straw yard to the hard concrete floor in the cubicle housing may have led to more sole lesions. However, welfare benefits of straw-bedded housing systems have been reported. For example, Fregonesi and Leaver (2002) reported that more synchronised lying was observed in straw yards compared to cubicle housing. Cows increase their lying time in straw yards probably because of the softer surface and more flexible space allowance compared to cubicles (Phillips and Schofield, 1994). Daily lying time and synchronised lying are important welfare indicators for cows (Miller and Wood-Gush, 1991).

It has been reported that prepartum cows showed a preference for lying down on a sand floor in the maternity pens compared to rubber mats, although both of the floors were bedded with deep straw (Campler et al., 2014). Campler et al. (2015) suggested that the housing system can affect the calving process. They found that cows that were housed in deep-bedded straw yards during the last four weeks before calving expelled calves faster after the first appearance of the feet of the calf, compared to cows that were in cubicle housing during the same period. It would appear that straw yards are a more suitable housing system for prepartum cows compared to cubicle housing, as long as a soft floor and clean bedding are provided. Provision of a softer floor for early lactation cows could also prevent cows from developing hoof problems after calving.

1.6. Dry period length

The main purposes of the dry period are to allow cows to recover after the previous lactation, maximise milk production during the subsequent lactation (Kuhn et al., 2006a; 2006b; Pezeshki et al., 2010) whilst maintaining or improving udder health when cows are not lactating (Bradley and Green, 2004; Bradley et al., 2011). However, in the beginning of the 2000s, the length of the dry period was reviewed (Grummer and Rastani, 2004), and the practice of shortening or even omitting the dry period has been proposed as a new strategy to simplify the nutritional management of cows during the dry period (Rastani et al., 2005). A "no planned dry period" system i.e. continuous milking up to the day of calving (Schlamberger et al., 2010) or until when milk yield declined below 2 kg/day (Rastani et al., 2005; de Feu et al., 2009) or 5 kg/day (Andersen et al., 2005) would reduce or eliminate stressful management practices such as an abrupt cessation of milking and frequent regrouping.

Van Knegsel et al. (2013) summarised the existing literature that investigated the effect of shortening or omission of the dry period. They reported that milk production decreased by 1.4 kg/day and 5.9 kg/day after cows experienced a short dry period (28-35 days) and a "no planned dry period", respectively, compared to a traditional dry period length (53-63 days). Pezeshki et al. (2010) reported that a non-lactating interval enhanced redevelopment of mammary epithelial cells, suggesting that the omission of this period would lead to a reduction in milk secretion capability. However, it is argued that the loss of milk production due to a shortened length of dry period or continuous milking can be compensated for by additional milk production during the extended previous lactation period (Santschi et al., 2011a).

1.6.1. Effects on DMI and metabolic status

It has also been reported that a shortened or "no planned dry period" is associated with a lower incidence of metabolic disorders and better reproductive performance during the subsequent lactation period (Rastani et al., 2005). This would reduce the cost for veterinary treatment whilst increasing the amount of saleable milk. Shortening of the dry period length has been reported to improve postpartum DMI (28 days: Rastani et al., 2005) or postpartum DMI corrected by body weight (30 days: Gulay et al., 2003) compared to a conventional length of dry period of 56 and 60 days. Although de Feu et al. (2009) reported that "no planned dry period" did not affect postpartum DMI, improved energy balance was found in cows with shortened or "no planned dry periods" compared to cows with a conventional length of the dry period (Gulay et al., 2003; Rastani et al., 2005; Watters et al., 2008; de Feu et al., 2009; Klusmeyer et al., 2009).

The majority of studies on dry period length focus on the postpartum performance of cows, but some studies have investigated the effects of energy intake and metabolism in dry cows. For example, improved DMI has been reported in prepartum cows with a short dry period (28 days: Rastani et al., 2005) or "no planned dry period" (Andersen et al., 2005; Rastani et al., 2005; de Feu et al., 2009) compared to cows with a traditional dry period length (seven weeks: Andersen et al., 2005; eight weeks: de Feu et al., 2009; 56 days: Rastani et al., 2005). Prepartum energy status in cows with shortened or no dry periods was better than cows with a traditional dry period length (Andersen et al., 2005; Rastani et al., 2005; Watters et al., 2008; Schlamberger et al., 2010). However, a different length of the dry period appeared to have no effect on prepartum BCS (Pezeshki et al., 2007; Santschi et al., 2011b). Moreover, the dry period shortened to 35 days (Soleimani et al., 2010) or 30 days (Gulay et al., 2003) did not affect prepartum DMI and BCS.

1.6.2. Health disorders

The number of cows used in the studies reported in the literature is usually too small to detect any effect of dry period length on the incidence of health disorders (e.g. Gulay et al., 2003; Pezeshki et al., 2008). However, a retrospective study reported that a dry period longer than 60 days increased the incidence of dystocia (Atashi et al., 2013). In controlled studies, associations between shortened dry periods and health disorders are contradictory. Watters et al. (2008) reported that different length dry periods (55 days vs 34 days) did not affect the incidence of peripartum diseases and culling rate within 30 days in milk (DIM). However, Santschi et al. (2011c) found that multiparous cows with a conventional dry period (60 days) had a higher incidence of ketosis and higher culling rate during the first 30 days of lactation compared to cows with shortened dry period (35 days). Similarly, Schlamberger et al. (2010) reported that cows continuously milked until calving had a lower incidence of hypoglycaemia and a lower risk of ketosis, compared to cows with a dry period of 56 days.

1.6.3. Effects on new IMI

Pezeshki et al. (2010) pointed out that continuous milking improves udder health as teats are cleaned every day and a shortened dry period allows cows to be dried off at lower levels of milk production, which can reduce the risks of new IMI. Many studies measured somatic cell count (SCC) as a parameter for the risk for mastitis, and most of the results showed no effect of dry period length on SCC (Gulay et al., 2003; Rastani et al., 2005; Church et al., 2008; Watters et al., 2008; Butler et al., 2010; Schlamberger et al., 2010). In contrast, Klusmeyer et al. (2009) reported that shortening the dry period from 60 days to 32 days decreased SCC, whereas omission of the dry period resulted in the highest SCC during the subsequent lactation period. Similarly, Andersen et al. (2005) found increased SCC in continuously milked cows as parturition approached. These studies, however, did not investigate any associations between increased SCC and the incidence of clinical mastitis.

1.6.4. Effects on colostrum quality and calf birth weight

When cows were continuously milked, there was a reduction in the quality of colostrum (concentration of IgG and protein in the first milk: Rastani et al., 2005; Klusmeyer et al., 2009). Mammals start secretion of immunoglobulin around 10 days prior to parturition (Wheelock et al., 1965), and so continuous milking may prevent immunoglobulin accumulation in the udder, and dilute the immunoglobulin concentration in colostrum. In contrast, shortened dry period lengths appear to have no effect on the quality of colostrum (Rastani et al., 2005; Watters et al., 2008) and calf birth weight (Gulay et al., 2003; Rastani et al., 2005; Pezeshki et al., 2008; Watters et al., 2008).

1.6.5. Difference between parities

Some studies have suggested that the negative effects of a shortened dry period length on subsequent lactation were more prominent in primiparous cows (Annen et al., 2004; Pezeshki et al., 2007; Santschi et al., 2011b). Annen et al. (2004) also reported that IgG concentrations in the first milked colostrum of multiparous cows were not different between 60-day dry periods, 30-day dry periods, and “no planned dry period”. However, the same study found lower concentrations of colostrum IgG in primiparous cows with “no planned dry period” compared to primiparous cows that had 30- or 60-day dry periods. Since the mammary gland of cows continuously develops until the second lactation, some authors have suggested that primiparous cows would require a longer dry period to sustain mammary growth and functionality (Annen et al., 2004) and to promote turnover of mammary epithelial cells (Pezeshki et al., 2007). Therefore, reduced dry period length systems may be more applicable to multiparous cows than to primiparous cows.

1.6.6. Overall benefits of shortened/no planned dry period

The omission of the dry period may mean that cows would no longer experience the stress associated with management procedures such as social mixing and diet changes during the late gestation period. Additionally, a no planned dry period system would enable farmers to reduce the management complications of relocating dry cows to a dedicated dry unit and feeding dry cow rations (Grummer and Rastani, 2004). Cows that were continuously milked had better prepartum DMI and possibly better metabolic status (lower NEFA, BHB, higher glucose), which could suggest that cows may have sufficient nutrients to maintain body reserves as well as nourish the developing fetus. Indeed, no difference was found in the birth weight of calves from either system. To date, the long term impacts on the health, welfare and productivity of calves have not been fully investigated. Further research is needed to evaluate the effect of continuous milking during late gestation on cows and developing fetuses.

1.7. Effect of maternal experience on fetal development

The previous sections summarised conventional dry cow management practices and other environmental factors that could affect the health and welfare of cows. Most of these management practices aim to maximise milk production after calving, but some of them can act as a stressor for dry cows. It has been reported in humans and laboratory animals that maternal stress during pregnancy can affect fetal development (Braastad, 1998; Weinstock, 2008). Boksa (2010) also reported that maternal infection and immune system activation during the prenatal period can impair offspring brain development, which has both acute and life-long effects on offspring behaviour.

Compared to humans and laboratory animals, studies relating to this area in farm animals are limited. However, existing literature has shown that there are some associations between maternal experience during pregnancy and offspring survival, health and productivity (Arnott et al., 2012; Rutherford et al., 2012). Kamal et al. (2014) reported that a younger maternal age at calving, a higher level of milk yield during pregnancy and a shorter dry period were all associated with lighter calf birth weight. Black et al. (2017) also suggested potential associations between maternal confinement or forced exercise during late pregnancy and offspring activity levels and restlessness during weaning. These findings would suggest that it is possible that certain maternal factors can affect fetal development. Additionally, lower body weight at birth or during the neonatal period is associated with an increased risk of mortality (McCorquodale et al., 2013) and morbidity (Windeyer et al., 2014), meaning that maternal factors can also affect offspring survival.

Dairy calves are usually separated from their dam immediately after birth and artificially reared by producers. Their environment is far from natural, and they are expected to adapt to these artificial environments from an early age. Therefore, having calves with better adaptability and ability to cope with stress would be advantageous. Potential consequences of maternal stress during the whole gestation period on offspring growth and survival have been previously reviewed by Arnott et al. (2012) for both beef and dairy cattle. Therefore, the following section will focus more on stress experienced during late pregnancy, and stress related to management during the dry period. The information on cattle in this area is still limited, and so relevant studies on other farm animal species will be included.

1.7.1. Maternal nutrition

In human studies, it has been reported that maternal obesity is associated with health and behavioural problems in offspring (O'Reilly and Reynolds, 2013). In dairy cows, over-conditioning at calving is not recommended because of its negative consequences on calving ease and postpartum health and productivity (Grummer, 1995), but Osorio et al. (2013) reported that maternal overfeeding could also affect offspring metabolism and immune responses. They found some evidence that a maternal high-energy diet (1.47 Mcal/kg) during the last three weeks before calving resulted in lower birth weight, lower insulin sensitivity and lower anti-inflammatory states in offspring compared to a control diet (1.24 Mcal/kg). However, most of the results in the study showed marginal or no significant effects of the maternal diet, and so it is difficult to draw definite conclusions from these results.

In contrast, the effects of maternal undernutrition on restricted fetal growth, offspring health and welfare have been well documented in human epidemiological studies (Reynolds and Caton, 2012), and farm animals (Wu et al., 2006; Funston et al., 2010a; White and Windsor, 2012). Wu et al. (2006) reported that the effect of maternal undernutrition on fetal growth retardation was most prominent in late gestation, compared to maternal feed restriction in early to mid gestation. In cattle, the speed of fetal growth is highest during the last two months of gestation (Bell et al., 1995; Reynolds and Redmer, 1995), which corresponds to the dry period. Therefore, undernutrition during the dry period could be disadvantageous for fetal growth. Gao et al. (2012) also reported that low maternal energy intake during the last three weeks before calving reduced not only calf birth weight and body size (height and length), but there were also indications of lower immune system function.

Similarly, Funston et al. (2010a) reported that undernutrition in pregnant beef cattle resulted in compromised passive immune transfer, which increases disease risks in early postnatal life. Indeed, Quigley and Drewry (1998) reported that nutritional management during the dry period can affect colostrum quality (levels of immunoglobulin, energy, protein and vitamins), and consequently can affect offspring health and growth. Funston et al. (2010a) also reported that prenatal nutrition levels enhance muscle fibre development in the fetus, which affects postnatal growth and productivity. Effects of prenatal nutrition level on postnatal growth have been reported in out-wintered beef cattle (Martin et al., 2007; Larson et al., 2008; Funston et al., 2010b), where protein supplementation for out-wintered cows successfully increased birth weight and weaning weight compared to non-supplemented offspring.

1.7.2. Maternal social stress

The adverse effects of maternal social stress on offspring have been well documented in laboratory animals. For example, pregnant rats mixed with unfamiliar rats or kept in overcrowded conditions produced pups with lighter body weights compared to non-stressed rats (Zielinski et al., 1991; Brunton and Russell, 2010). Increased anxiety-related behaviour in the "elevated plus maze test" was also observed in rats born to dams that experienced social and physical stress during pregnancy (Bosch et al., 2007). Moreover, maternal social stress is associated with greater hypothalamic-pituitary-adrenal (HPA) axis responsiveness to acute stress in the offspring (Brunton, 2013). To my knowledge, the effects of maternal social stress on offspring body weight, growth and behaviour have not been investigated in cattle. However, research on other farm animal species has provided evidence of potential associations between maternal social stress and offspring performance.

For example, it has been reported that repeated social mixing during pregnancy in pigs did not affect offspring birth weight (Jarvis et al., 2006; Couret et al., 2009a; Rutherford et al., 2009). In contrast, artificially increased levels of maternal cortisol during pregnancy decreased piglet birth weight and weaning weight (Kranendonk et al., 2006). Kranendonk et al. (2007) also reported an association between maternal social rank during gestation and offspring growth and behaviour. This study found that piglets born to dominant sows (50%> success in displacement at feed station) had a higher weaning weight than piglets born to subordinate sows (50%< success in displacement at feed station). Piglets born to sows with lower maternal social rank were less active during a novel object test and took longer to approach the object. These findings would suggest potential associations between maternal social stress during pregnancy and offspring performance.

Jarvis et al. (2006) also reported that piglets born to sows that experienced social mixing during the second or third trimester of pregnancy were less able to cope with weaning stress compared to control piglets (born to unmixed sows). These piglets showed persistent aggressive behaviour towards littermates. Additionally, prenatal exposure to maternal social stress resulted in maladaptive maternal behaviours of daughter offspring, shown as a greater responsiveness to their piglets. This study also found that maternal social stress increased the responsiveness of the offspring's HPA axis to social mixing with unfamiliar pigs. Rutherford et al. (2009) reported that piglets born to sows that experienced social mixing during the second trimester of pregnancy exhibited more frequent pain-related behaviour after tail-docking (at three days of age) compared to piglets that experienced no prenatal social stress. The litter pain score was positively correlated with post-mixing maternal cortisol levels.

Sheep studies have shown that lambs born to ewes that experienced two occasions of social isolation (with or without the presence of a dog) during the last trimester were heavier at birth (Roussel et al., 2004) and at three months of age (Roussel-Huchette et al., 2008). Similarly, shearing ewes during early to mid-pregnancy resulted in heavier body weight of lambs at birth (Corner et al., 2007; Sphor et al., 2011). Maternal social isolation during late pregnancy increased basal serum cortisol levels in the lamb at 25 days of age compared to control lambs (Roussel et al., 2004), although this was not observed at one and three months of age (Roussel-Huchette et al., 2008) or eight months of age (Roussel et al., 2004). In contrast, behavioural observations at eight months of age showed that prenatally stressed lambs engaged more in exploratory behaviour and locomotor activities during the behavioural tests. This effect was not observed at 25 days of age. Similarly, Roussel-Huchette et al. (2008) reported that prenatal social isolation with the presence of a dog decreased the reactivity of lambs at three months of age in a fear-eliciting situation (startling stimulus test), with no difference in lambs at one month of age.

As discussed in the earlier section, dry cows can experience some degree of social stress because of group housing and a dynamic social environment. Therefore, it is possible that social stress during late pregnancy or the dry period could affect fetal development, as seen in other animal species. Pig studies have shown the potential negative impact of prenatal social stress on offspring, whilst some sheep studies reported potentially advantageous outcomes. Sheep studies have also shown that behavioural consequences appear to emerge later in life, suggesting that prenatal social stress could have a long lasting impact on offspring development.

1.7.3. Transportation

Previous studies on beef cows have investigated the effect of repeated transportation during pregnancy on fetal development (Lay et al., 1997a) and physiological responses of offspring to stressful procedures (restraint and hot-iron branding: Lay et al., 1997b). They found that repeated prenatal transportation tended to increase the body weight of fetuses at 266 days of gestation, and significantly increased the weight of the heart and pituitary gland relative to body weight (Lay et al., 1997a). The level of serum cortisol after restraint was elevated for a longer period in calves born to dams repeatedly transported during pregnancy compared to control calves (no prenatal transportation). In these studies, stress treatment was repeatedly applied to dams during early to mid pregnancy (60, 80, 100, 120 and 140 days of gestation), and the effect of late gestation stress was not investigated.

The sheep study by Roussel-Huchette et al. (2008) also investigated the effect of repeated transportation (ten times) of ewes during the last six weeks of gestation on offspring performance. They found that body weight of offspring at birth, one and three months of age was not affected by prenatal transportation stress. However, behavioural tests at one and three months of age showed that lambs born to ewes repeatedly transported during pregnancy were less fearful of a novel test arena, a novel object and startling stimuli, compared to non-prenatally stressed lambs. The studies above show contrasting outcomes of repeated transportation during pregnancy, which may be due to species differences and/or different treatment periods. Dairy cows are often transported during pregnancy, and transportation could be a source of stress. However, it is unlikely that cows are transported as frequently as in these experiments. It would be worthwhile to investigate the potential effects of prenatal transportation on offspring in more industry relevant settings.

1.7.4. Maternal heat stress

Dairy calves born to dams experiencing prepartum heat stress had significantly lower birth weights (Collier et al., 1982; Wolfenson et al., 1988; Adin et al., 2009; do Amaral et al., 2009, 2011, Tao et al., 2011, 2012a). Collier et al. (1982) reported that heat stress during the last trimester decreased placental weight. In sheep studies, it has been reported that heat stress during gestation reduced placental weight (Bell et al., 1989) and total uterine umbilical blood flow (Dreiling et al., 1991; Reynolds et al., 2006), which resulted in lower fetal body weight. Tao and Dahl (2013) argued that there are various factors adversely affecting calf birth weight, including retarded placental development, lower nutritional intake of dams, and also shortened length of the gestation period due to heat stress (do Amaral et al., 2009, 2011, Tao et al., 2011, 2012a).

Tao et al. (2012a) investigated the long-term effects of maternal heat stress on heifer calves, following them until 7 months old. Weaning weight of prenatally heat-stressed calves was significantly lower than calves born to dams under cooling treatments. However, prenatal cooling treatment did not affect body weight gain from birth to weaning. After weaning, body weight and wither height were not different between groups. Calves in this study were given their dam’s colostrum, which was not different in quality (Ig level). However, lower serum IgG was observed in calves born to heat stressed cows compared with calves born to cooled cows, which suggests that prenatal heat stress might compromise the absorption of colostrum immunoglobulins. Additionally, the level of plasma total protein during the first 28 days of life was lower in calves born to heat stressed cows compared to calves born to cooled cows.

Interestingly, this study found that plasma cortisol levels at calving were not different between cows under heat stress and under cooling treatment. Similarly, prenatal heat stress did not affect the concentration of serum cortisol in heifer calves. However, a tendency for higher cortisol concentrations was found in prenatally cooled heifers at birth. The authors argued that prenatal heat stress might have reduced the reactivity of the HPA axis in response to stress during calving. However, many studies in rodents have reported that prenatal stress increases the reactivity of the HPA axis in offspring (Bosch et al., 2007; Brunton, 2013), which is mediated by excessive fetal exposure to glucocorticoids (Kapoor et al., 2006). The effect of prenatal heat stress on the stress responsiveness of the HPA axis in offspring requires further investigation.

1.7.5. Maternal health

In human epidemiological studies, it has been reported that prenatal maternal infections increase the risk of psychiatric and neurologic disorders in offspring (Boksa, 2010). Laboratory rodent models confirm that experimental infections during pregnancy alter maternal placental function and the central nervous system in offspring. This change resulted in behavioural problems such as deficits in learning ability and social interactions (Boksa, 2010). However, Meyer et al. (2007) discussed in their review that infections during the early stages of gestation can have more severe effects on fetal brain development compared to infections during the last trimester of gestation. If this is the case, health conditions in dry cows during late pregnancy might not have major impacts on foetal neurodevelopment.

In beef cattle, it has been reported that calves born to heifers treated for two internal parasites (nematodes and liver fluke) during pregnancy had a higher birth weight compared to calves born to heifers that received no treatment (Loyacano et al., 2002). This is most likely due to improved maternal nutritional status, as heifers treated for parasites had a higher body condition score. Dry cow therapy in pregnant beef cows (intramammary infusion of antibiotics at 2-5 months of pregnancy) has been shown to not only successfully reduce the incidence of dry period mastitis and somatic cell counts during the subsequent lactation, but also improve calf growth until weaning (Lents et al., 2008). However, it is not clear from this study whether improved calf growth was because of improved maternal health (lower incidence of mastitis) or improved udder health and milk production after calving.

In dairy cows, Lundborg et al. (2003) investigated maternal factors that could affect calf birth size (heart girth measurement at birth), morbidity and growth during the first 90 days of life. They found that smaller calf size was associated with an increased incidence of mastitis in the dam in the last 49 days before calving. A higher incidence of respiratory disease from birth to 90 days of age was observed in calves born to cows that had disease from 280 to 50 days before calving. Similarly, the incidence of retained placenta at calving in the cow was associated with higher risks of respiratory disease in calves. The incidence of retained placenta is associated with compromised prepartum energy status of cows (Huzzey et al., 2011; Chapinal et al., 2012). Therefore, these results indicate that prepartum maternal health and energy status could affect offspring morbidity. This study also found that a higher incidence of respiratory disease in the calf was associated with a shorter dry period. Interestingly, there was no association between the incidence of diarrhoea and any maternal factors.

1.7.6. Dystocia

Dystocia i.e. delayed or difficult parturition (Lombard et al., 2007) is caused by various factors such as fetal malpresentation, mismatch of maternal-offspring size, non-genetic and genetic factors (Lombard et al., 2007) including calving season, age at calving, and nutritional management (Johanson and Berger, 2003; Steinbock et al., 2003; Mee, 2008). It has been reported that dystocia resulted in a reduction in milk production and greater weight loss in cows during the early lactation period (Berry et al., 2007). Moreover, dystocia is associated with higher circulating cortisol levels in calves at birth (Civelek et al., 2008; Barrier et al., 2013). Lower vigour (Barrier et al., 2013) and increased morbidity and mortality rates in dairy calves (Lombard et al., 2007; Henderson et al., 2011; Barrier et al., 2013) have also been reported as a consequence of dystocia.

Dystocia is an event associated with the birth of the calf, and therefore, is probably not directly related to prenatal stress. However, it is an important factor affecting the welfare of cows and calves. As mentioned in the previous section, Campler et al. (2015) reported that prepartum housing systems could affect the calving process. The same study found that an earlier onset of some neonatal behaviours (standing up and suckling) was observed in calves born to dams housed in straw-bedded yards compared to calves born to dams housed in a cubicle shed. Management practices that promote easier calving processes would be of potential benefit to both cows and calves.

1.8. Conclusion

Despite a considerable amount of research into dry cow management, the majority of the research focus has been concentrated on the improvement of health and productivity in the cow during the early lactating period. In relation to changes in management practices around dry-off and the physical and social environments of dry cows, a limited number of issues related to behaviour and welfare have been addressed. Additionally, potential links between prepartum maternal health, stress and nutritional conditions and offspring performance have been highlighted in studies on cows and other farm animal species. Some of the outcomes may be disadvantageous for artificially reared dairy calves. The number of studies investigating maternal-offspring relationships in dairy cows remains limited. However, research on this topic would provide insights for the development of management systems that would improve not only maternal performance, but also the future performance of their offspring from the prenatal stage onwards.

Despite research interest into social stress in dairy cows, to my knowledge, the consequences of social stress on fetal development and subsequent effects on offspring health, behaviour and welfare have not been investigated. Therefore, this project aims to investigate the impact of management practices in late pregnancy on offspring health, behaviour and welfare. The first objective is to develop an understanding of common dry cow and pre-weaned calf management systems on commercial dairy farms. This will help uncover knowledge gaps between research and industry, and identify the potential challenges to dry cows and pre-weaned calves associated with current management practices. The second objective was to investigate the effect of management practices in late pregnancy on the health and welfare of offspring under commercially relevant conditions.

To achieve the first objective, a survey was conducted among dairy farmers in the United Kingdom (**Chapter 2**). To achieve the second objective, two experiments were conducted. The first experiment investigated the effect of high stocking density during the dry period on the health, behaviour, metabolism and stress physiology of cows, and on the health and behaviour of calves during the pre-weaning period. The second experiment investigated the impact of prenatal exposure to cold weather on the health and behaviour of calves in the first two weeks of life.

The hypotheses of these experiments were that:

1) High stocking density during the dry period would result in more frequent agonistic social interactions, altered feeding and lying behaviour, the activation of physiological stress response and negative energy balance in the cows (**Chapter 3**).
2) Maternal exposure to high stocking density during the dry period would reduce offspring body weight and vigour, impair offspring health and growth, and increase offspring behavioural and physiological responses to challenges during the pre-weaning period (**Chapter 4**).
3) Maternal exposure to winter weather during pregnancy would reduce offspring body and weight, impair offspring health and growth, and increase offspring behavioural responses to challenges in the first two weeks of life (**Chapter 5**).

Chapter 2 :

A survey of dry cow and pre-weaned calf management on UK dairy farms

2.1. Introduction

In dairy production, the dry period (non-lactating interval before calving) is considered necessary to maximise milk production during the subsequent lactation period (Collier et al., 2012). The importance of good management during the dry period has been well accepted, and there has been an increase in the amount of research on management during this period, especially the close-up dry period in the three weeks pre-calving (LeBlanc et al., 2006). The focus of such research has been the development of dry cow management systems that optimise postpartum performance and reduce transition cow disease. As a result, it is widely accepted that management practices during the dry period play an essential role in maximising milk production without compromising health and fertility during the subsequent lactating period. On the other hand, more and more attention has been paid to farm animal welfare, and the importance of understanding and meeting the behavioural, biological and physiological needs of farm animals has become well recognised.

Some studies have pointed out potential sources of stress associated with dry cow management, and their potentially negative consequences for the health, welfare and production of dairy cows (Zobel et al., 2015). For example, the process of drying-off itself can cause discomfort for high yielding cows due to increased udder pressure (Bertulat et al., 2013, Tucker et al., 2009). Feed restriction is commonly conducted to prevent over-conditioning at calving but could cause distress and hunger (Valizaheh et al., 2008). Social regrouping is often performed from late gestation to the early lactation period (Cook and Nordlund, 2004), which has been shown to increase competition at the feed-face (Lobeck-Luchterhand et al., 2014). Overstocking also increases aggressive interactions (Huzzey et al., 2006), which are associated with stress (Huzzey et al., 2012). Moreover, reduced prepartum feed intake, compromised metabolic status or higher faecal cortisol levels have been associated with increased risks for postpartum diseases (Huzzey et al., 2007, 2011; Ospina et al., 2010a; Chapinal et al., 2011).

The studies described above indicate potential sources of stress for cows during the dry period and their potentially negative consequences for the health, welfare and production of dairy cows. Additionally, associations between maternal stress during pregnancy and offspring performance have been reported in many mammalian species including laboratory animals (Braastad, 1998; Weinstock, 2008) and farm animals (Arnott et al., 2012; Rutherford et al., 2012). Although information on dairy cows is still limited, there is the potential that stressful maternal experiences could affect offspring health and welfare (Arnott et al., 2012). Rearing healthy replacement heifers is a strategy to maximise future profits on dairy farms.

However, the artificial rearing of the pre-weaned calf involves procedures associated with poor welfare such as early separation from the mother (Flower and Weary, 2003), individual housing (de Paula Vieira et al., 2012b) and feed restriction (Soberon et al., 2012), which could affect future profit.

Despite considerable research highlighting potential welfare problems of dry cow and pre-weaned calf management, there is limited information on current management practices on commercial dairy farms. Conducting a survey of farmers is a way to develop an understanding of typical management practices on commercial dairy farms, which would assist researchers in assessing knowledge gaps between research and dairy farms, and identifying further research priorities. Therefore, a survey was performed among dairy farms in the United Kingdom (UK) to investigate the prevalence of dry cow and pre-weaned calf management practices, focusing on those practices that might result in potentially stressful experiences for the cows and calves.

2.2. Materials and Methods

2.2.1. Questionnaire design

A survey of UK dairy farmers was conducted from November 2014 to April 2015. An online and paper questionnaire were created using Snap® survey software (Snap version 11). Consultation on a draft of the questionnaire was sought from members of AHDB Dairy and the National Farmers Union of Scotland, and the questionnaire was modified based on the feedback received. The questionnaire was then piloted by a farm manager and technicians at SRUC's Dairy Research and Innovation Centre and Langhill Farm (University of Edinburgh) to assess the effectiveness of the questionnaire and to estimate the time to complete. This led to minor adjustments of the questionnaire. The final questionnaire (see **Appendix**) contained 35 questions divided into the following six sections:

1) General information: the first section (Q1-Q9) included general questions about the farm including herd size, milk production, calving season, peak calving month, dry cow grouping strategy and maximum group size.

2) Dry-off procedure: the second section (Q10-Q14) included questions about routine management procedures around dry-off, the level of milk production at dry-off, and procedures designed to reduce milk production. Two questions regarding the reduction in milk production led certain participants to sub-questions for more detailed information.

3) Feeding management: the third section (Q15-Q19) covered practices relating to feeding, which included questions on types of feed used for late lactation and dry cows, delivery methods of concentrates, the frequency of fresh feed delivery and push-up schedules for dry cows. Participants were also asked if all dry cows had access to the feed face simultaneously.

4) Housing systems: the fourth section (Q20-Q23) covered housing systems and types of housing used for cows during the late gestation period in summer and in winter. Farmers' perception of stocking density was also measured by asking participants to choose either a photograph (straw yards) or the number (cubicle sheds) that they thought was the most appropriate stocking density. According to the requirements of the UK Red Tractor Assurance for Farms - Dairy Standards (2014), the minimum space allowance for cows (700 kg body weight) kept in straw or sand yards is 5.75 m^2 per cow. Photographs of cows in a straw yard at 105% (5.4 m^2/cow), 100% (6.0 m^2/cow), 95% (6.7 m^2/cow) and 90% (7.5 m^2/cow) stocking densities were displayed in the questionnaire. For a cubicle shed, the minimum requirement is at least one cubicle per cow. The question asked how many cows were appropriate for a cubicle shed with 100 cubicles with answer choices of 105, 100, 95 and 90 cows.

5) Calf management: the fifth section (Q24-Q30) covered calf management during the pre-weaning period. This section was designed to develop an understanding of the typical experience of pre-weaned calves on commercial dairy farms. Questions included the timing of separation from dams, housing systems, milk feeding management, weaning age and dehorning procedure.

6) Information about respondents: the final section (Q31-Q35) gathered demographic information about respondents e.g. gender, age and level of experience in dairy cattle management. The final questions asked participants to rank the three most important management periods in a cow's life from five stages of the production cycle: young stock, fresh calver/early lactation, mid to late lactation, far-off dry and close-up dry period.

2.2.2. Distribution

The online survey opened on the 16th November 2014, and was first promoted via social media sources and newsletters from The Dairy Research and Innovation Centre (SRUC) and the Dairy Herd Health and Productivity Service (Royal (Dick) School of Veterinary Studies, University of Edinburgh) with a short description of the background and the purpose of this survey. Additionally, emails were sent to farmers' associations, agricultural colleges, large animal veterinary practices and dairy milk purchasers to ask if the survey could be promoted in their magazines to their members. The Royal Association of British Dairy Farmers (RABDF: Kenilworth, UK), the Organic Research Centre (Newbury, UK), Myerscough College (Preston, UK) and Shepton Vets (Shepton Mallet, UK) agreed to introduce the survey in their newsletters or via their social media.

Flyers and a paper version of the survey were distributed to dairy farmers at a number of events for dairy farmers, including Agri-Scot (November 2014), the Royal Highland Show (June 2015) and the Livestock Show (July 2015). Eight hundred and fifty copies of the paper survey were distributed by mail to members of the RABDF along with their quarterly magazine in April 2015.

2.2.3. Data processing and analysis

Data from the returned paper surveys were entered into Snap® survey software to merge online and paper responses and the results then exported to Excel 2013 for analysis. Quality and coherence of answers to each question were checked and inadequate responses (e.g. multiple answers to questions that required a single response, answers with one digit missing) were deleted, or were edited to make sense where the meaning was clear. A total number (n) used for percentage calculations was reported when it was different from the total response. This happened when questions were asked to specific respondents and when questions were not answered by all respondents.

Some of the respondents answered with a range although a single value was expected (Q9: n=1, Q10: n=11, Q11a: n=5, Q25a: n=11, Q25b: n=15, Q27: n=8, Q28: n=3 and Q29: n=35). These answers were adjusted either by calculating averages where an average was required (Q25b and Q27), by taking the highest value given where a maximum number was expected (Q9) or by taking the lowest value given where it was considered as most suitable (Q25a). The median value was used for analysis of the questions that asked neither maximum/minimum value nor averages (Q10, Q11a, Q28 and Q29).

In Q 12, 62 respondents (41.9%) gave answers as percentages when the actual number of cows was expected. The number of cows was calculated for those farms by multiplying the ratio (percentages/100) with the total number of lactating cows (all lactating cows/heifers + dry cows), obtained from Q1. In Q13b, a single answer was expected but one respondent ticked two answers,"1-2 days" and "3-4 days". The answer "3-4 days" was chosen as this can represent the possible longest duration for cows to experience a dietary change.

In Q20 and Q21, many respondents did not provide a valid response for the second part of the questions: '*if cows are kept both inside and outside, please indicate whether cows are free to move in and out, or they are moved by farm staff at set times'* (valid response rate: Q20=30.1%; 20/65 responses, Q21=17.1%; 6/35 responses). Therefore, this part of the question was not included in data analyses. In the final question (Q35), 128 respondents ranked each period as 1, 2 and 3, whilst the remaining 19 respondents either just ticked the three periods or chose multiple periods as the same rank. Therefore, the answers were analysed with or without taking ranks into account.

Counts and percentages were calculated for all factors from the survey data and corresponding tables and bar charts generated using Excel 2013. Summary statistics were produced using Minitab 17 (Minitab Ltd, Coventry, UK). For all variables, means, standard deviations (SD), medians, interquartile ranges (IQR), minimums, maximums and number of observations were calculated and histograms generated. Spearman's rank correlation was used to examine associations between two continuous variables, and a Kruskal Wallis test was used to compare values between more than two groups.

2.3. Results

A total of 148 respondents completed the survey (online: 27, event: 15, RABDF: 106). This represents approximately 1.1 % of the total dairy farm population in the UK in 2015 (dairy producer numbers=13,570: AHDB Dairy, 2017c). The majority of respondents were male (91.2%, n=135) and were predominantly farm owners (69.6%). The majority of respondents had more than 20 years of experience in managing dairy cattle (73.7%), and the most common age range was between 45 and 64 (57.4%). Demographics of respondents are summarised in **Table 2.1**.

Table 2.1. Demographics of respondents, indicated as the percentage (number) of respondents**.**

Age		**Experience**		**Role in the farm**	
16-24	3.4 (5)	**<2 years**	2.0 (3)	**Employed stock person**	1.3 (2)
25-44	31.8 (47)	**2-5 years**	5.4 (8)	**Family member**	17.6 (26)
45-64	57.4 (85)	**6-10 years**	8.8 (13)	**Manager**	11.5 (17)
65+	7.4 (11)	**11-20 years**	10.1 (15)	**Owner**	69.6 (103)
		>20 years	73.7 (109)		

2.3.1. General information about farm

The average number of lactating cows on respondent farms, including dry cows and lactating heifers, was 283 cows (±242 SD; median=220, IQR=134-345, range: 50-2000). Frequency distributions of the number of lactating cows and the number of dry cows are indicated in **Figure 2.1**. The total number of cows on respondent farms was 41,841 cows, and 88% of the cows were from farms with a herd size greater than 150 cows. The average annual milk sale of respondent farms was 2,227,469 litres (±2,336,019 SD), and the annual average milk sold per cow of respondent farms was 8,623 litres/cow/annum (±2156 SD). On average, first lactating heifers accounted for 23.7% (±7.0 SD) of the lactating herd on respondent farms and non-milking heifers (>6 months) accounted for 27.7% (±10.2 SD) of the whole herd. Frequency distributions of the percentages of lactating heifers and non-milking heifers are indicated in **Figure 2.2**.

Respondents used seven different breeds of cow (**Table 2.2**). Holstein, Friesian and Holstein-Friesian (HF) were the most common breed, used by 86.5% of farms. A small number of farms (2.7%, n=4) used multiple breeds of cow, and 7.5% (n=11) used cross-bred cows such as HF × Swedish Red × Montbéliarde, HF × Jersey and HF × Brown Swiss or others. Ninety-seven percent of farms (n=143) bred their own replacement heifers (breed all: 88.4%, n=130; buy in some: 8.8%, n=13), and only 2.7% (n=4) bought all of their replacement heifers in.

The majority of farms (64.2%, n=95) calved all year round, while the remaining farms (n=51) had cows calving for between 2 and 11 months (average: 6.2 months). No relationship was found between overall herd size and the number of months when calving occurred (r_s=-0.079, p=0.34). A figure of monthly calving distribution (**Figure 2.3**) displays a slight bimodal distribution with two peaks appearing in early spring and autumn. One hundred and seven farms (72.3%) chose a single month of the year when they had most cows calving, showing that 52.3% (n=56) of these farms calved between July and September (**Figure 2.3**).

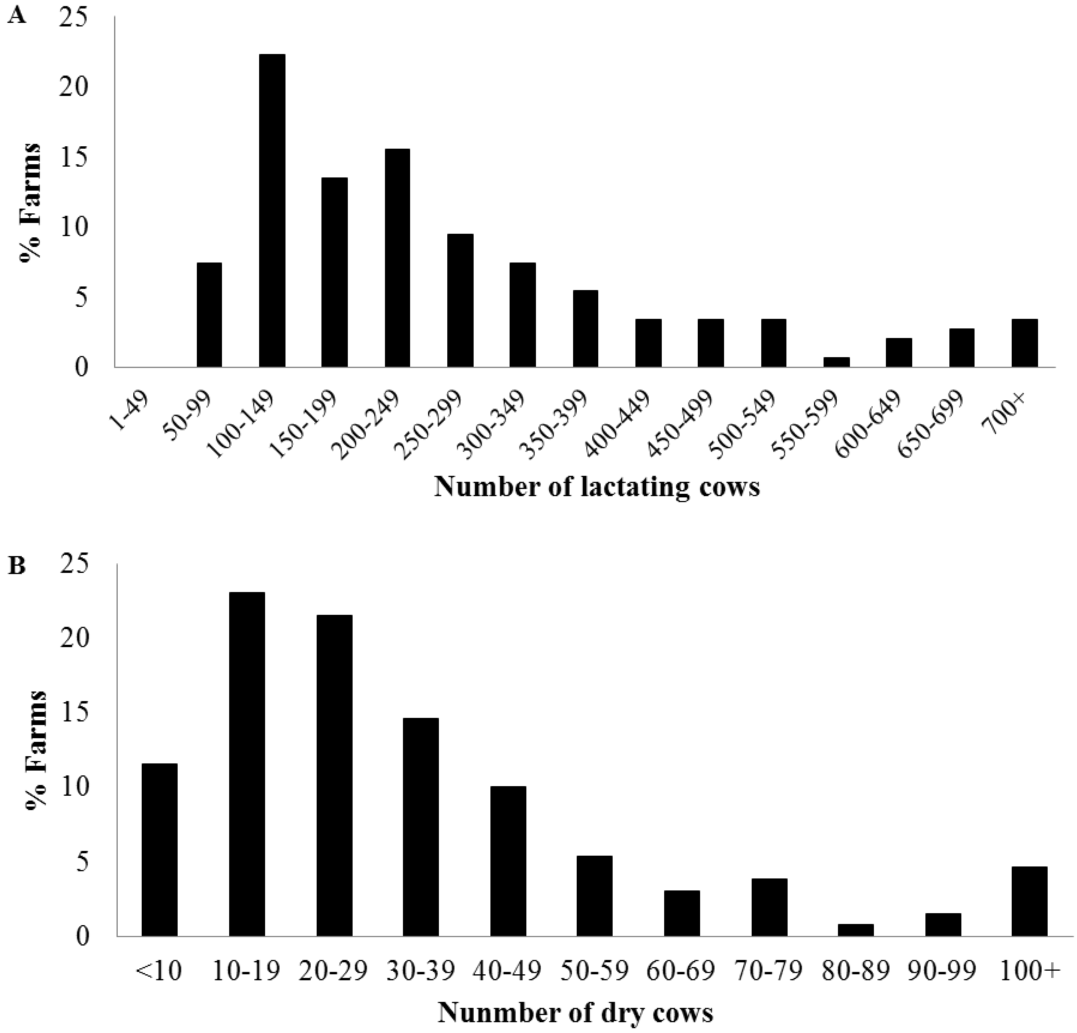

Figure 2.1. Percentage distributions of respondent farms (n=148) grouped by the number of lactating cows (A: lactating cows + lactating heifers + dry cows) and dry cows (B).

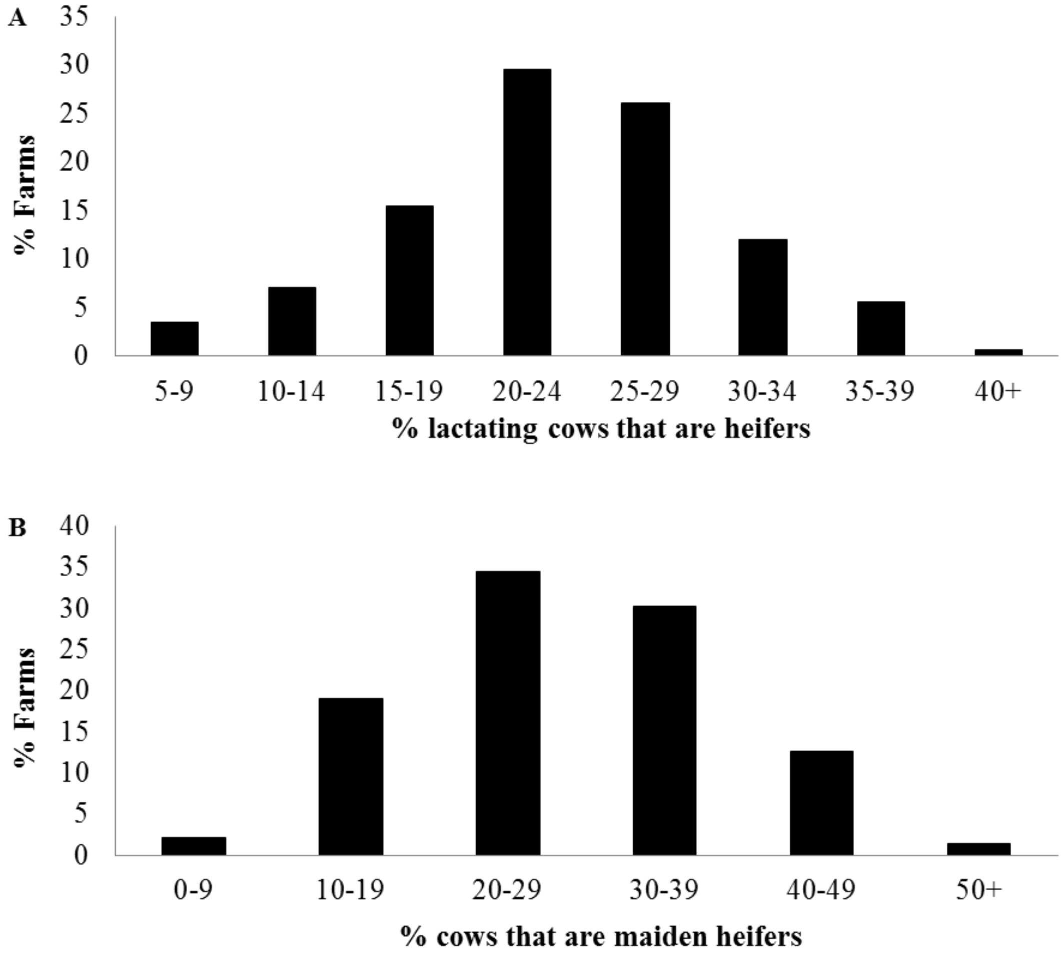

Figure 2.2. Percentage distributions of respondent farms grouped by the percentage of lactating cows that are heifers (A), and the percentage of all cows in the herd that are maiden heifers (B).

Table 2.2. Breeds of cow used by respondent farms.

Breeds	% (n) Farms†
Holstein Friesian	86.5 (128)
Jersey	4.7 (7)
Montbéliarde	0.7 (1)
Ayrshire	1.4 (2)
Brown Swiss	1.4 (2)
Norwegian Red	0.7 (1)
Cross	7.4 (11)

†multiple answers possible

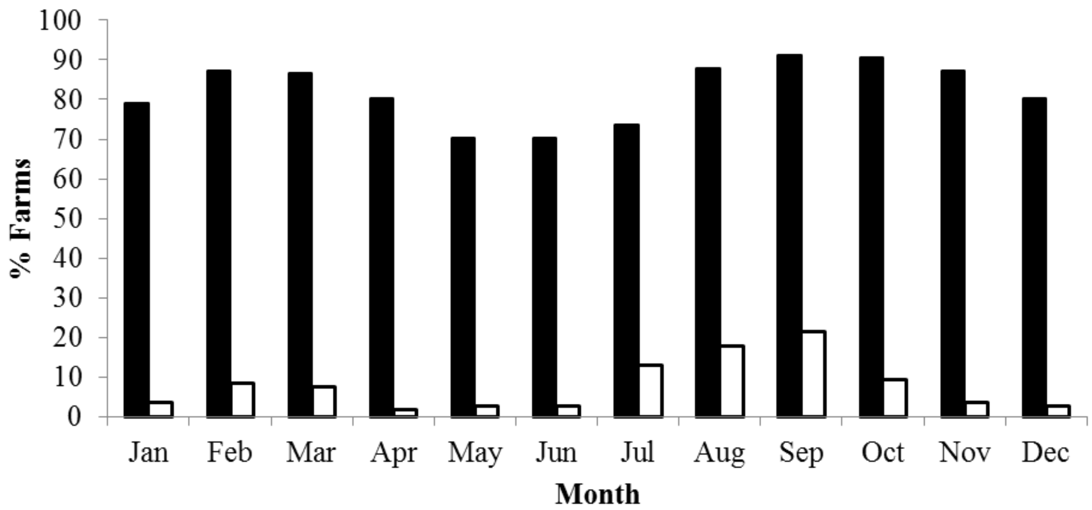

Figure 2.3. Percentage distribution of respondent farms grouped by calving month. Black bars indicate months of the year cows calved (n=148), and white bars indicate a peak calving month of 107 farms.

2.3.2. Grouping strategy

The majority of farms (73.0%, n=108) managed dry cows in two groups, dividing cows according to the stage of gestation (far-off and close-up groups), whilst 25.7% of respondent farms (n=38) had a single dry group. Only 4.1% of farms (n=6) had a separate group for fat cows. In addition to these management groups, 4.1% of farms (n=6) had "other" management groups, where dry cows were grouped for example based on calving season, health condition and the level of milk production during the previous lactation period. Pregnant maiden heifers were most often mixed with adult cows before first calving (80.7%, n=107), and only 14.5% of farms (n=21) mixed after calving. However, there were 4.1% of farms (n=6) that never mixed maiden heifers/first lactation cows with adult cows.

On the majority of farms (84.2%, n=123), dry cows were kept in dynamic social groups, and 65.9% of them had cows calving all year round. On 23 farms, the dry cows remained in the same social group either always (5.5%, n=8) or when possible (10.3%, n=15). These 23 farms consisted of 7 farms that have only a single dry group, and 16 farms that have two dry groups. Sixty one percent of these farms (n=14/23) had cows calving all year round. The median number of lactating cows for the farms that had a stable social grouping system was 215 (range: 92-2000).

The percentage distributions for the maximum size of the dry cow groups are described in **Figure 2.4**. A wide variation was found in the maximum group size for dry cows (4-400 cows), but the majority of farms had a maximum group size of less than 50 cows (single dry group: 70.3%, n=26/37; far-off dry group: 66.7%, n=70/105; close-up dry group: 80.2%, n=85/106). Positive correlations were found between overall herd size and the maximum group size for dry cows (single dry group: r_s=0.75, p<0.001; far-off dry group: r_s=0.69, P<0.001; close-up dry group: r_s=0.57, P<0.001). The number of months when cows were calving and the maximum dry cow group size were negatively correlated (single dry group: r_s=-0.55, P=0.001; far-off dry group: r_s=-0.51, P<0.001; close-up dry group: r_s=-0.50, P<0.001).

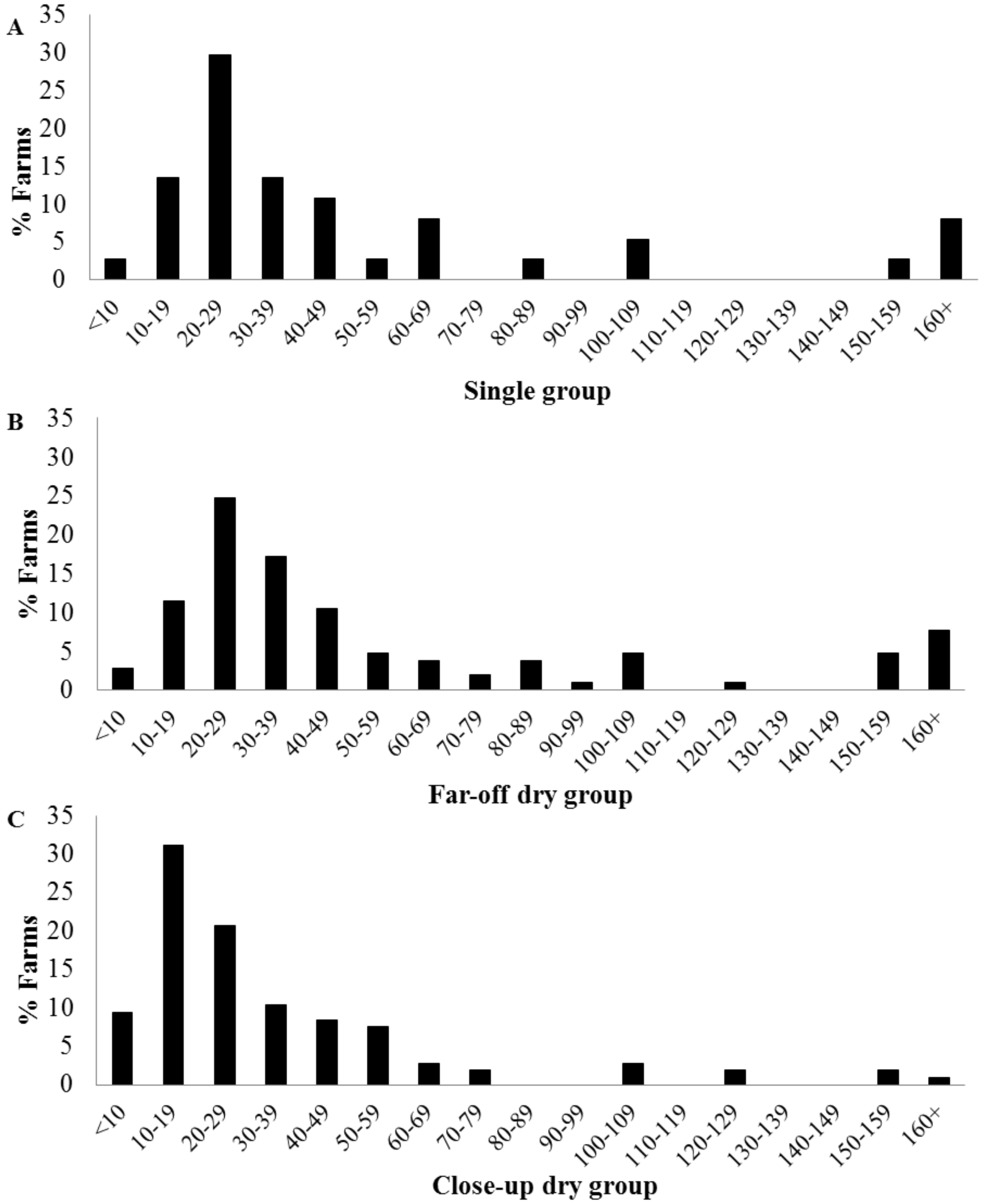

Figure 2.4. Percentage distributions of respondent farms grouped by the maximum group size for each of the dry cow management groups (A: Single group, B: Far-off dry group, C: Close-up dry group).

2.3.3. Dry-off procedure

The percentage distribution of dry period length for respondent farms is shown in **Figure 2.5**. The median length of the dry period was 56 days (IQR=50-60, range: 30-90). Only 5.6% of farms (n=8) dried cows off ≥65 days before their expected calving date, while 9.8% of farms (n=14) continued milking until <45 days before the expected calving date. There was no correlation between overall herd size and the length of the dry period (r_s=-0.10, P=0.28). The majority of respondents (95.9%, n=140/146) used antibiotic dry cow intramammary tubes at dry-off, and an internal teat sealant was used by 82.2% of farms (n=120). Twelve percent of farms (n=17) used an external teat sealant, including those using both internal and external teat sealants (n=13). Antibiotic intramammary tubes were mainly used in combination with internal or external sealants (82.2%, n=120/146), but 13.7% of farms (n=20) used antibiotic intramammary tubes only. There were 4.1% of farms (n=6, including one organic farm) that did not use antibiotic intramammary tubes at dry-off.

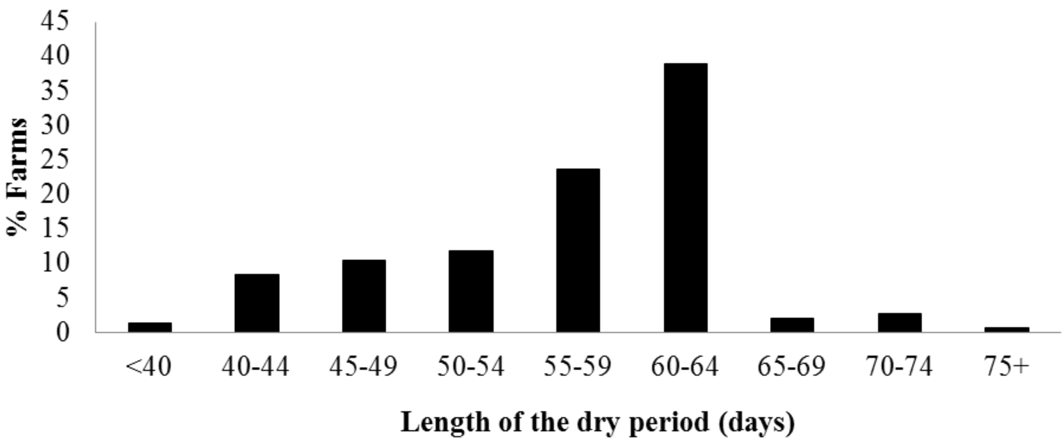

Figure 2.5. Percentage distribution of respondent farms grouped by the length of dry period.

One hundred and sixteen farms provided information on how they stopped milking at dry-off. The majority of farms (83.6%, n=97/116) stopped milking abruptly. Seventeen percent of farms (n=20) reduced the frequency of milking to once a day, including 1.4% of farms (n=3) that further reduced the frequency of milking from once a day to every other day. The length of the period of reduced milking varied from 1 day to 60 days, with a median of seven days.

Almost half of the farms (46.6%, n=68) used a dietary change to reduce milk production before dry-off, with 35.6% doing this for all cows, and 11.0% just for high yielding cows. The most common change in diet to reduce milk production at dry-off was to reduce the quantity of concentrates (**Figure 2.6**). The other common strategies were to stop all concentrate feeding, or to reduce the quantity of the milking cow ration fed (**Figure 2.6**). Additional dietary changes at dry-off included the addition of hay/straw to silage, or feeding hay/straw only, an alternation of silage types with or without a change in quantity (**Figure 2.6**). This altered dietary management at dry-off continued for 7 days or longer on the majority of farms (75.0%, n=51/68), but some farms continued it for shorter periods of 3 to 4 days (13.2 %, n=9/68), or 5 to 6 days (11.8%, n=8/68).

On average, 46.7% (n=16,553) of cows on respondent farms (total number of cows=35,450) were producing 10-20 kg of milk at dry-off, while 26.2 % (n=9,295) produced less than 10 kg of milk. The remaining 27.1 % (n=9,602) produced more than 20 kg at dry-off, and 61.8% (n=5,931) of these high yielding cows were abruptly dried-off, which represents 16.7% of the total cows included in this survey. Most of the cows from the farms implementing intermittent milking produced either <10 kg/day (n=2079) or 10-20 kg/day (n=2160), while 285 cows produced >20 kg/day at dry-off.

Farms with higher milk sales and a higher average milk yield per cow were more likely to have a higher percentage of cows that produced >20 kg/day of milk at dry-off (annual milk sales: r_s=0.31, P<0.001; average milk yield per cow: r_s=0.61, P<0.001). In contrast, a significant negative correlation was found between the percentage of cows produced <10 kg/day of milk at dry-off and the annual milk sales (r_s=-0.23, P=0.005) or the average milk yield (r_s =-0.599, P<0.001). No significant association was found between herd size and the percentages of cows that produced <10 kg/day (r_s=-0.04, P=0.620), 10-20 kg/day (r_s=-0.160, P=0.056) or >20 kg/day (r_s=-0.131, P=0.121) of milk at dry-off.

Another procedure that was often performed around dry-off was foot trimming. Seventy seven percent of respondent farms (n=112) routinely had their cows hoof trimmed around dry-off, with 42.5% (n=62) doing so before dry-off, 26.0% (n=38) on the day, and 6.2% (n=9) after. The remaining 2.1% of farms (n=3) had their cows hoof trimmed at various times before, on the day, or after dry-off.

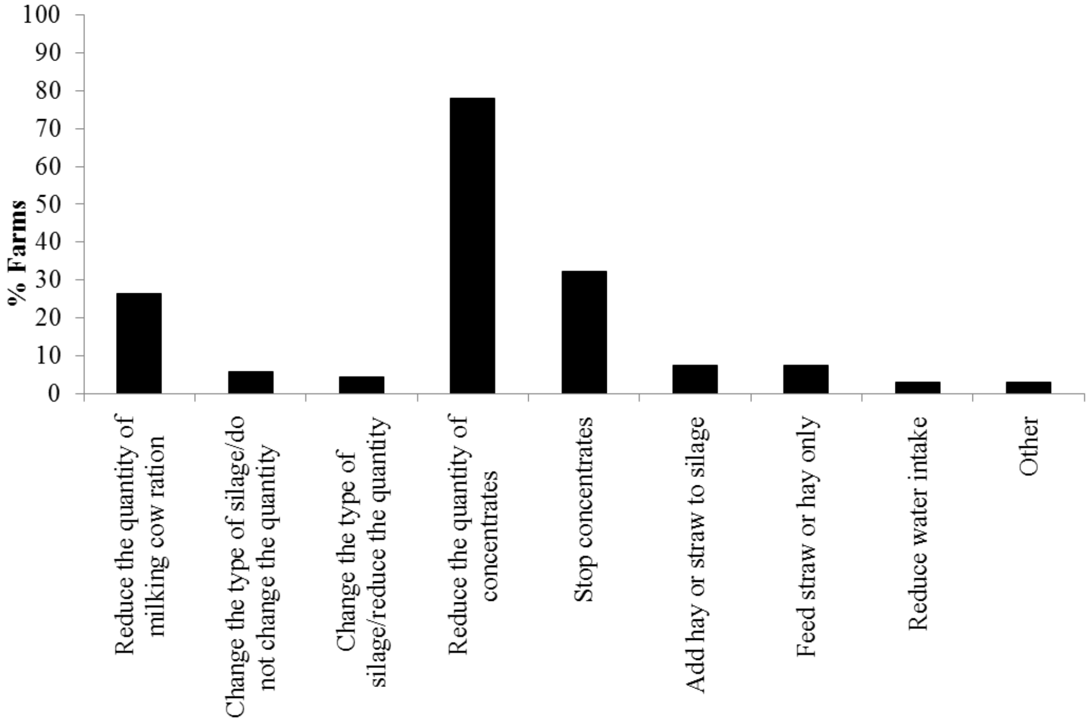

Figure 2.6. Types of dietary change to reduce milk production at dry-off among 68 farms. *(multiple answers possible)*

2.3.4. Feeding management

Feedstuffs used for late gestation dairy cows are summarised in **Table 2.3**. Grass silage was the most common type of forage fed to cows during the late gestation period, followed by maize and whole-crop/arable silage. The percentage of farms that fed straw and hay increased from the late lactation to far-off dry period, whilst the percentage of farms that fed silage, sources of energy and protein declined. From the far-off to close-up dry period, a slight increase was seen in the percentage of farms that fed whole-crop/arable silage, maize silage and sources of energy and protein. From the close-up dry to calving period, the percentage of farms feeding silage, energy and protein sources increased, while the percentage of farms that fed straw declined.

All of the respondent farms changed their cows' diet at least once during the late gestation period (from the late lactation to calving period). Most of these dietary changes happened between the late lactation and far-off dry period (81.3%, n=109/134), while 55.6% (n=74/133) of farms changed the diet between the far-off and close-up dry period, and 47.2% (n=68/144) changed from the close-up to calving dry period. It was common to manage cow groups based on their nutritional requirement according to their gestation stage, but 37.0% (n=40/108) of farms did not change the diet for dry cows even though they had two management groups.

Table 2.3. Percentages (n) of respondent farms that used the following feedstuffs for their cows during the late lactation, far-off dry, close-up dry and calving period.

	Cow management groups			
	Late lactation	**Far-off dry**	**Close-up dry**	**Calving**
No. of respondents†	**n=139**	**n=139**	**n=144**	**n=143**
Straw	38.8 (54)	64.0 (89)	68.1 (98)	49.7 (71)
Hay	4.3 (6)	15.1 (21)	14.6 (21)	15.4 (22)
Grass silage	94.2 (131)	73.4 (102)	77.1 (111)	90.9 (130)
Whole-crop/ Arable silage	22.3 (31)	15.1 (21)	20.8 (30)	28.0 (40)
Maize silage	46.8 (65)	25.2 (35)	40.3 (58)	44.8 (64)
Cereals, Starch/ Other source of energy	61.2 (85)	28.1 (39)	46.5 (67)	60.8 (87)
Concentrates/ Other source of protein	71.9 (100)	35.3 (49)	65.3 (94)	82.5 (118)
Mineral/ Vitamin supplement	80.6 (112)	77.7 (108)	83.3 (120)	86.0 (123)

†multiple answers possible

Table 2.4 summarises the frequency of fresh feed delivery and feed push-ups for each of the management groups. Regardless of the grouping strategy for dry cows, fresh feed was most often delivered to dry cows once a day, while 6.8% of farms (n=10) reported that they delivered feed less than every second day. Half of respondent farmers reported that they did not push feed up for dry cows because of the feed bunk design (50.7%, n=74/146), and a small number of farms never pushed feed up for dry cows (2.7%, n=4). Twelve farms changed the frequency of feed push-ups when the group was changed from far-off to close-up. This included five farms that increased the frequency of feed push-ups from once or twice daily to three times or more. The other seven farms changed the frequency of feed push-ups because of a change in feed bunk design (e.g. change from “no need to push up” to “three or more times”, or vice versa). For cows from late lactation to calving, the majority of respondent farms fed concentrate or non-forage supplements mixed with silage/forage

(**Figure 2.7**). The rest of the farms mainly fed concentrate either on top of silage/forage or fed it separately (e.g. in the milking parlour for late lactation cows). The majority of respondents (86.0%, n=123) reported that all of their dry cows were able to feed at the same time after fresh feed was delivered, while 14.0% of farms (n=20) reported that not all of their dry cows were able to feed at the same time.

Table 2.4. Frequency of feed delivery and feed push-ups for dry cows in different management groups.

	Single group % (n)	**Far-off % (n)**	**Close-up % (n)**	**Fat cow/Other % (n)**
How often do you deliver fresh feed to cows in the following groups? (n=147)				
No. of respondents†	**n=37**	**n=107**	**n=107**	**n=12**
Twice daily	10.8 (4)	1.9 (2)	1.9 (2)	8.3 (1)
Once daily	67.8 (25)	81.3 (87)	85.0 (91)	66.7 (8)
Every second day	10.8 (4)	12.1 (13)	8.4 (9)	16.7 (2)
Less than every second day	10.8 (4)	4.7 (5)	4.7 (5)	8.3 (1)
How often do you push feed up for dry cow groups? (n=146)				
No. of respondents†	**n=37**	**n=106**	**n=106**	**n=12**
Three or more times a day	5.4 (2)	23.6 (25)	29.2 (31)	8.3 (1)
Twice daily	27.0 (10)	16.0 (17)	9.4 (10)	16.7 (2)
Once daily	10.8 (4)	12.3 (13)	10.4 (11)	0 (0)
Never	0 (0)	3.8 (4)	3.8 (4)	0 (0)
No need to push up because of the feed bunk design	56.8 (21)	44.3 (47)	47.2 (50)	75.0 (9)

†multiple answers possible

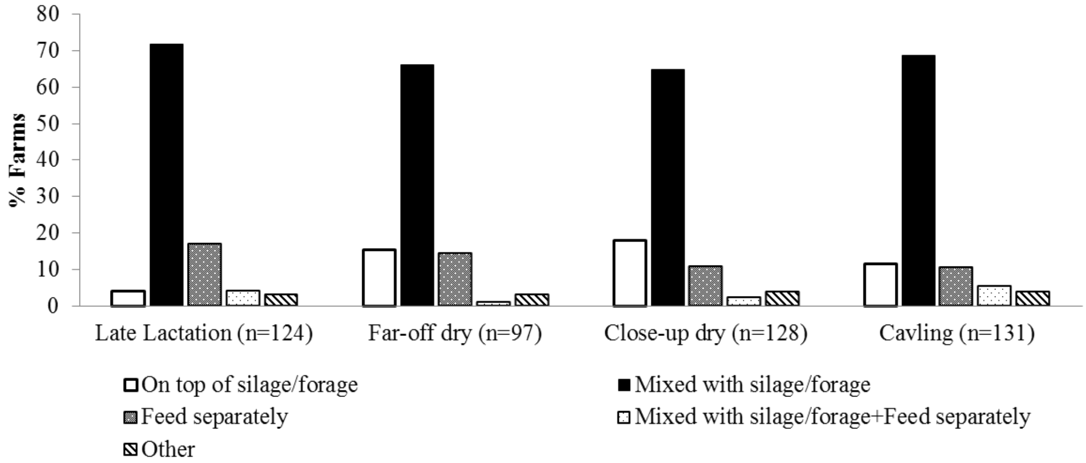

Figure 2.7. Percentage of farms that gave concentrate or non-forage supplement in each of the above methods to cows in each of the gestation stages.

2.3.5. Housing systems and environment

Housing systems and environment for late gestation cows in summer and in winter are summarised in **Table 2.5**. Approximately 70.0% of farms did not house their late lactation and far-off dry cows, and approximately 60.0% of farms kept their close-up and calving cows inside only. However, there were still more than a third of farms that kept their close-up and calving cows exclusively outside. Only a few farms used both inside and outside housing systems for their late lactation and dry cows in summer. In winter, over 92.0% of farms kept cows exclusively inside for all four periods, and less than 7.0% of farms kept their late gestation cows exclusively outside. Only a few farms kept cows outside during winter. The percentages of farms that used both inside and outside housing systems were also low at 2.8% (n=4) for their late lactation and far-off dry cows, 2.1% (n=3) for close-up cows and 0.7% (n=1) for calving cows.

The types of housing used for late gestation cows are also shown in **Table 2.5**. The most common environment for late lactation cows and far-off dry cows in summer was pasture/paddock, followed by cubicle housing. Although no farmers used straw/sand yards for their late lactation cows, 8.6% of farms used straw/sand yards for their far-off dry cows. In winter, late lactation cows were predominantly kept inside in cubicles, and far-off dry cows were also mainly kept inside in cubicles or straw/sand yards. Close-up and calving cows were predominantly kept inside in straw/sand yards.

Table 2.5. Housing system and environment for late gestation cows in summer and winter.

	Cow management groups			
	Late lactation % (n)	**Far-off dry % (n)**	**Close-up dry % (n)**	**Calving % (n)**
Summer housing/environment				
No. of respondents	**n=132**	**n=140**	**n=137**	**n=136**
Inside only	22.0 (29)	25.7 (89)	56.9 (78)	60.3 (82)
Outside only	68.9 (91)	72.1 (101)	38.7 (53)	34.6 (47)
Both	9.1 (12)	2.1 (3)	4.4 (6)	5.1 (7)
Winter housing/environment				
No. of respondents	**n=141**	**n=139**	**n=142**	**n=139**
Inside only	92.2 (130)	93.5 (130)	95.8 (136)	97.1 (135)
Outside only	5.0 (7)	6.5 (9)	2.1 (3)	2.2 (3)
Both	2.8 (4)	0 (0)	2.1 (3)	0.7 (1)
Housing/environment in summer				
No. of respondents	**n=132**	**n=140**	**n=137**	**n=136**
Cubicles	22.0 (29)	17.1 (24)	11.7 (16)	5.1 (7)
Straw/sand yard	0.0 (0)	8.6 (12)	43.1 (59)	53.7 (73)
Pasture/Paddock	68.9 (91)	72.1 (101)	38.7(53)	34.6 (47)
Cubicles+ Pasture/Paddock	9.1 (12)	2.1 (3)	0.0 (0)	0.7 (1)
Straw/sand yard+ Pasture/Paddock	0.0 (0)	0.0 (0)	4.4 (6)	3.7 (5)
Other	0.0 (0)	0.0 (0)	2.2 (3)	2.2 (3)
Housing/environment in winter				
No. of respondents	**n=141**	**n=139**	**n=132**	**n=139**
Cubicles	79.4 (112)	52.5 (73)	24.6 (35)	14.4 (20)
Straw/sand yard	10.6 (15)	35.3 (49)	67.6 (96)	79.9 (111)
Cubicles+ Straw/sand yard	1.4 (2)	4.3 (6)	2.8 (4)	2.2 (3)
Cubicle+Straw/sand yard Pasture/Paddock	2.8 (4)	0.0 (0)	2.1 (3)	0.7 (1)
Pasture/Paddock	5.0 (7)	6.5 (9)	2.1 (3)	2.2 (3)
Other	3.0 (4)	1.5 (2)	0.7 (1)	0.8 (1)

Figure 2.8 and **Figure 2.9** indicate farmers' perception of an appropriate stocking density in cubicle sheds and straw yards, respectively. The majority of respondents (71.5%) chose the photograph showing 95% (6.7m^2/cow) or 90% (7.5m^2/cow) stocking density as appropriate for straw yards. Similarly, the majority of respondents (84.7%) selected 90% or 95% stocking density as appropriate for cubicle sheds. Approximately 30.0% of respondents selected the photo of 100% stocking density (6m^2/cow) or more for straw yards, while 15.4% selected 100% stocking density or more as appropriate for cubicles.

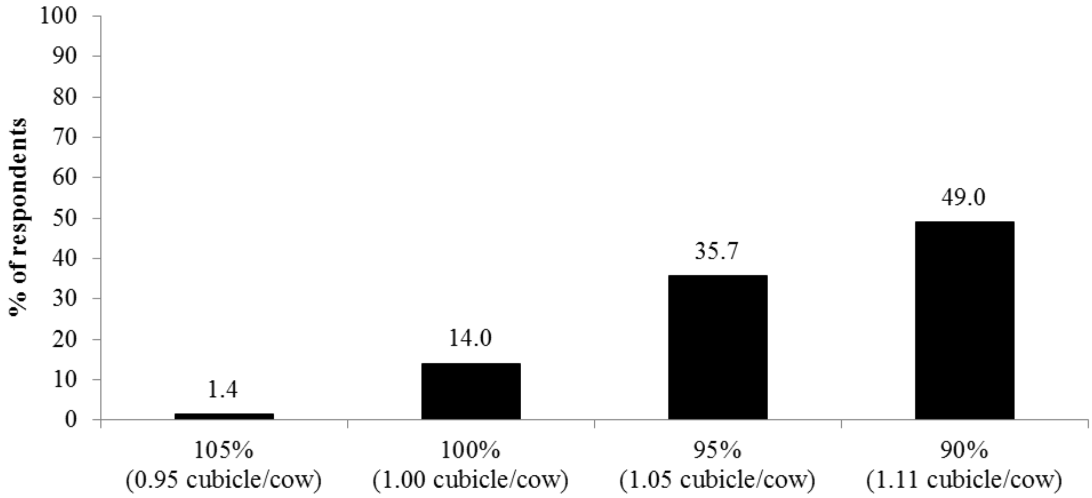

Figure 2.8. Respondents' opinion about appropriate stocking density for dry cows in 100 cubicles.

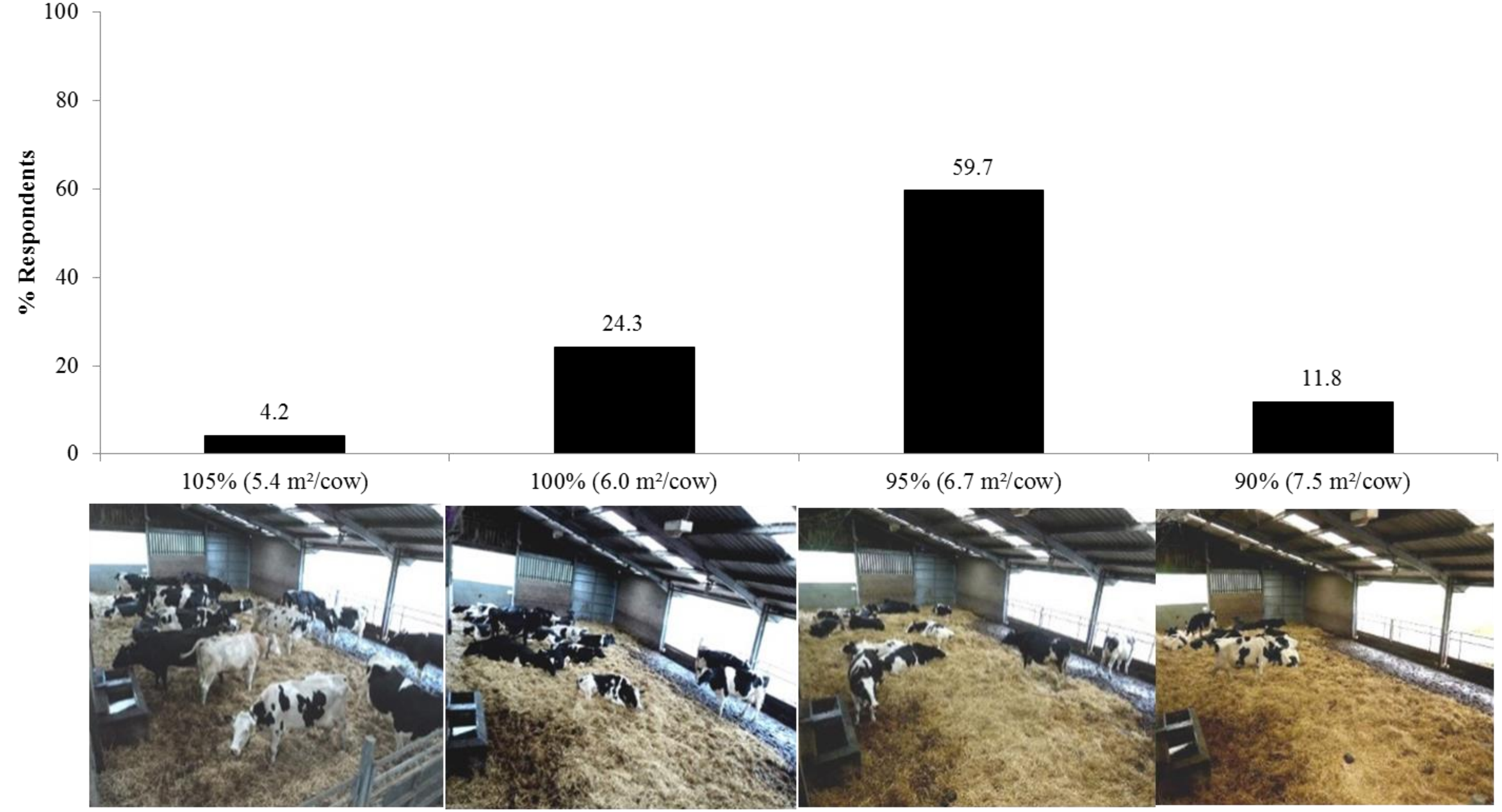

Figure 2.9. Respondents' opinion about appropriate stocking density for dry cows in straw yards.

2.3.6. Calf management

The majority of respondents (82.4%, n=122) separated calves from their dams within 24 hours after birth, with 29.0% of respondents (n=43) separating at less than six hours and 53.4% (n=79) separating between 6 and 24 hours. Only 17.6% of farms (n=26) kept calves with their dams for longer than one day. A significant difference in the median herd size was found among the timings of separation from dams (H=22.6, *df*=2, p<0.001). Calves from smaller sized farms (median herd size=133) were more likely to stay with their dams longer than one day after birth, and calves from larger sized farms (median herd size=300) were more likely to be separated from dams within six hours after birth. The median herd size for the farms that kept calves with dams between 6 and 24 hours after birth was 220.

The most common housing system for pre-weaned calves was a combination of individual and group housing systems (40.8%, n=60), where calves were kept for a certain period in individual hutches/crates and then moved to a group pen. On the other hand, 36.7% of farms (n=53) used only group housing, and 22.0 % (n=33) used only individual housing from birth to weaning. One farm answered "other", using group housing for the first ten days and then moving calves to individual housing until weaning. There was no difference in herd size among the types of housing used for pre-weaned calves (H=0.9, *df*=2, p=0.64). The median youngest age when calves were moved to group housing during the pre-weaning period was ten days old, with a wide range from two to forty days of age. The most common age for movement was between 7 and 13 days of age, but calves were also likely to be kept in individual housing for more than three weeks (**Figure 2.10**). The average group size for pre-weaned calves was 10.6±9.0 SD animals (median=8, IQR=5-15, range: 2-50).

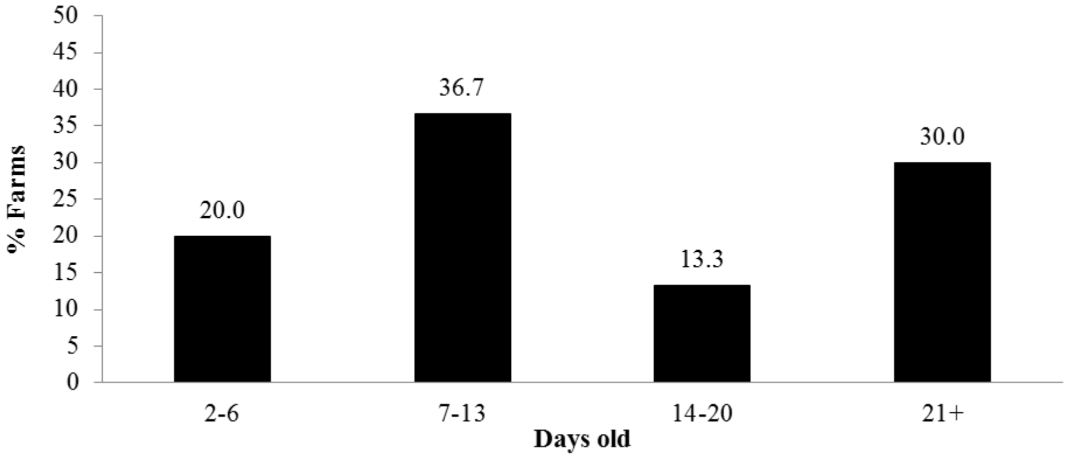

Figure 2.10. Time period in days during which calves were kept in individual hutches/crates using subset of 60 respondent farms that used both individual and group housing systems for calves during pre-weaning period.

More than half of the respondent farms (51.4%, n=75) provided four litres or less milk to their pre-weaned calves per day, followed by 39.7% of farms (n=58) giving five to seven litres of milk per day. The percentage of farms that gave eight litres or more milk was 6.8% (n=10), and only 2.1% of farms (n=3) allowed *ad libitum* milk intake for their pre-weaned calves. There was no difference in herd size among farms with different milk allowances for calves (H=0.27, *df*=2, p=0.87).

Although large variations were found in the age at weaning (**Figure 2.11**) and the length of the weaning process (**Figure 2.12**), eight weeks old was the most common age of weaning (IQR=8-10, range: 5-15 weeks old) and weaning typically took place over seven days (IQR=3-7, range: 0-21 weeks). Abrupt weaning was practised by 18.6% of farms (n=27).

The majority of farms used hot iron for disbudding (97.3 %, n=142) with local anaesthetic (98.6%, n=140), while 2.7% of farms (n=4) used caustic paste to disbud calves at one week of age. The median age of hot iron disbudding was four weeks old (IQR=3-6, range: 1-16 weeks old).

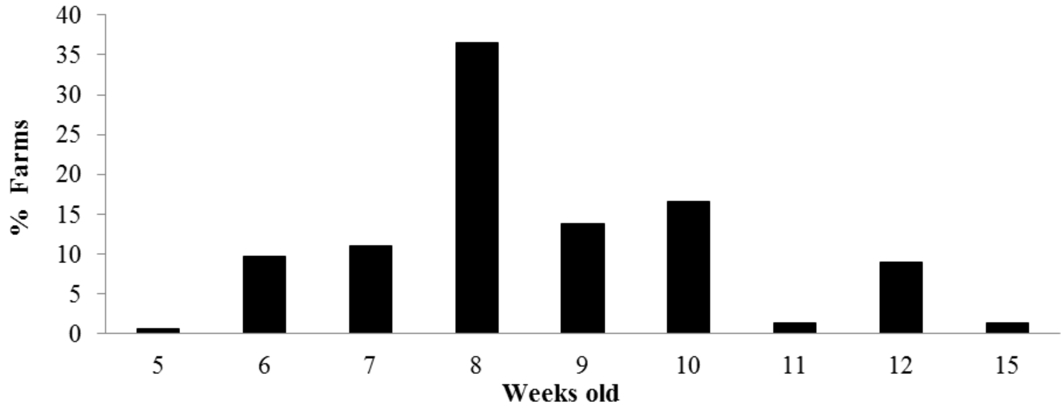

Figure 2.11. Percentage distributions of respondent farms grouped by age (weeks) at which calves are normally weaned off milk.

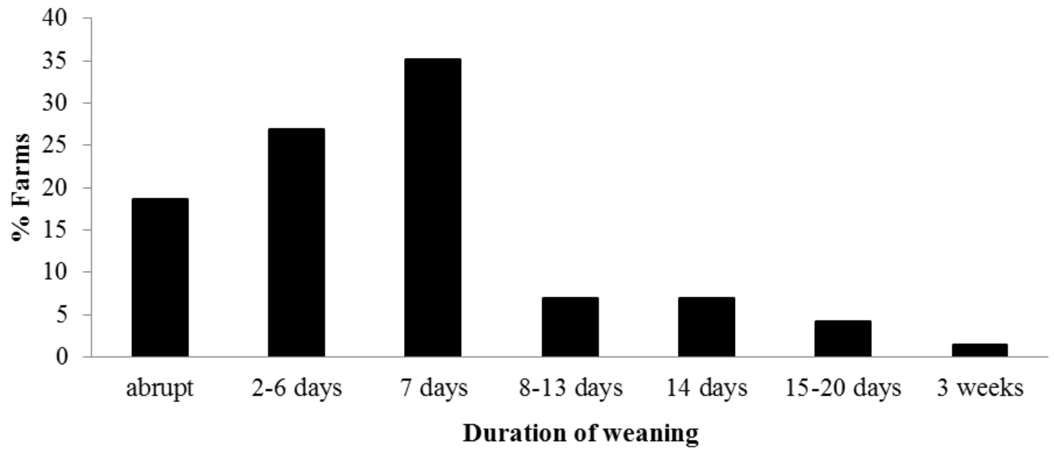

Figure 2.12. Percentage distribution of respondent farms grouped by duration of weaning process.

2.3.7. Perception of three most important periods in dairy cattle management

The close-up dry period and the fresh calver/early lactation period were most commonly selected as one of the three most important periods in dairy cattle management, followed by young stock (**Figure 2.13**). When ranking was taken into account (n=128), the close-up dry period was chosen as the most important period by 36.1% of respondents (n=47), whereas 37.5% (n=48) ranked it as the second most important period. The fresh calver/early lactation period was chosen by 39.8% of respondents (n=51) as the second most important, whereas 33.6% (n=43) considered this period as most important. Young stock was chosen as the third most important period by 33.6% of respondents (n=43), but this period was also ranked as the most important (21.9%, n=28) and the second most important (13.3%, n=17) period by other respondents in dairy management.

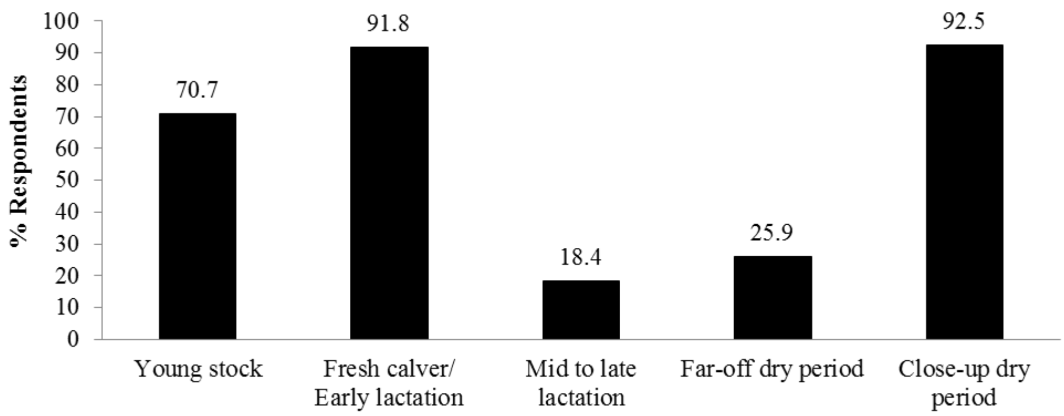

Figure 2.13. Percentages of farmers choosing the three most important periods in dairy cattle management (three choices were possible).

2.4. Discussion

DEFRA's annual statistics on the structure of the agricultural industry in the UK (DEFRA, 2015) reported that 53.6% of dairy holdings had a herd size of 50 or more cows (50-150 adult cows: 34.4%, 150+ cows: 19.6%). All of the respondent farms from the current survey had more than 50 lactating cows, and the farms with more than 150 lactating cows accounted for 70% of farms. The average herd size (including lactating cows, lactating heifers and dry cows) was bigger than the UK average (=140 adult cows per herd: AHDB Dairy, 2017a), and the average annual milk sales (the amount of milk sold per cow per year) of farms in this survey was also higher than the UK average (7,844 litres/cow/annum in 2014/2015: AHDB Dairy, 2017b). This suggests that the results from this survey may be more relevant to larger commercial farms. However, DEFRA (2015) reported that almost 95% of dairy cows in the UK are on farms with a herd size bigger than 50 cows, and 55% of them are on farms with 150 or more cows, showing that the majority of cows in the UK live on larger farms. Therefore, this survey is likely to represent farms in the UK that hold the majority of dairy cows.

2.4.1. General herd management

The most common breed of cow was Holstein-Friesian, found on 87% of farms, and the majority of farms had a single breed. The majority of farms had cows calving all year round, which was also found in a previous survey conducted in the UK (Haskell et al., 2007). Peak calving seasons in the UK appears to be in early spring and autumn, suggesting that the number of dry cows could be relatively high in mid-summer and mid-winter.

2.4.2. Grouping strategy

In this survey, the majority (84%) of farms kept dry cows in dynamic social groups (i.e. where cows were added and/or removed regularly), and only 6% of farms always kept them in the same social group. Frequent regrouping has been reported to increase aggressive social interactions (Cook and Nordlund, 2004), decrease dry matter intake (DMI) (Schirmann et al., 2011) and milk production in lactating cows (von Keyserlingk et al., 2008). A decrease in prepartum DMI and prepartum negative energy balance (i.e. higher concentrations of circulating nonesterified fatty acids) were associated with increased risks for postpartum disorders (Huzzey et al., 2007, 2011; Goldhawk et al., 2009; Chapinal et al., 2011). Therefore, prepartum social environment plays an important role in feeding activity of dry cows, affecting postpartum health.

It has been reported that cows that were most affected by regrouping were those with lower social rank (Olofsson, 1999; DeVries et al., 2004; Collings et al., 2011), and that heifers and primiparous cows are likely to suffer more from regrouping than multiparous cows, because they are normally in lower social rankings (González et al., 2003). The unfavourable effects of mixing primiparous cows with adult cows could be avoided by allowing heifers to acclimatise to a new environment with adult cows before their first lactation. Compared with heifers mixed after calving, heifers mixed with multiparous cows prior to calving were involved in fewer agonistic interactions after joining a lactating group (Boyle et al., 2013; de Vries et al., 2015). Barker et al. (2007) also found lower mean locomotion scores among pregnant heifers mixed with dry cows, compared to heifers mixed with lactating cows. The current survey showed that maiden heifers were most commonly mixed with dry cows in late pregnancy, suggesting that the drawbacks of mixing heifers with lactating cows after their first calving appear to be widely recognised by farmers. Additionally, in a small number of farms, maiden heifers or first lactating cows were never mixed with adult cows.

If calving periods are concentrated into blocks of time, many cows can be dried-off at the same time and moved as a group rather than as individuals. This may reduce the frequency of the introduction and removal of dry cows into the social group. In this survey, the respondent number was too small to investigate any relationship between the months when calving occurred and social structures for dry cows, but the results suggest that a socially stable grouping system could be achievable in herds with all year round calving. Indeed, such a system was actually practised on 17 farms. It would be interesting to investigate these farms further about how they achieved socially stable dry cow pen management.

In addition to the continuous introduction/removal of cows, the majority (73%) of farms had two management groups for dry cows, suggesting that cows were regrouped in the middle of the dry period. Having two management groups is a strategy which enables the feeding of different types of diet to dry cows according to their stage of gestation, as nutritional requirements for close-up dry cows are higher than far-off dry cows (NRC, 2001; Beever, 2006; Dann et al., 2006; Vickers et al., 2013). Indeed, 63% of the farms that had two management groups also changed the diet from the far-off to close-up dry period, which could disrupt both social and feeding behaviour at the same time. However, there were 19 farms that had a single dry group which replied that they changed the diet for their dry cows in the middle of the dry period. This might be a misunderstanding of the questions asked, as it would be impossible in a loose housing system to feed different types of diet to cows in a single group.

The maximum group size for dry cows was associated with the overall herd size, where farms with a larger lactating herd were more likely to have larger dry cow groups compared to farms with a smaller lactating herd. The maximum group size for dry cows was also affected by the number of months when calving occurs. Farms that had concentrated calving periods were more likely to have larger dry cow group size compared to farms that had cows calving all year round. Telezhenko et al. (2012) found no effect of group size on competition at the feed bunk in dairy cows, but it has been reported in a pig study that fewer aggressive interactions were seen as group size increased (Andersen et al., 2004).

2.4.3. Dry-off procedure

In the current survey, 73% of respondents had a traditional dry period length (51-60 days), which has been considered necessary to optimise milk production during the subsequent lactation period. In recent years, potential benefits of shortening or even omitting the dry period have been proposed, which includes improved prepartum DMI and/or reduced risks for metabolic disorders after parturition (Gulay et al., 2003; Andersen et al., 2005; Rastani et al., 2005; Pezeshki et al., 2008; de Feu et al., 2009; Schlamberger et al., 2010). Although all of the studies above reported that the shortened/omitted dry period resulted in a reduction in milk production during the subsequent lactation, Santschi et al. (2011a) claimed that additional milk production during the previous lactation could compensate for this loss.

However, the current study found that very few farms implemented a dry period of less than 40 days. In contrast, a dry period longer than 60 days has been reported to increase milk production during the subsequent lactating period while increasing a risk for dystocia (Atashi et al., 2013). Another study reported that a cow’s life time milk production was significantly reduced if the dry period was extended more than 70 days (Kuhn et al., 2006a). The current survey also found that dry periods longer than 60 days were not common.

The dry period is considered to be a critical time in the control of mastitis (Bradley et al., 2011). Antibiotic dry cow therapy (antibiotic infusion into the mammary gland at dry-off) is used to eliminate existing infections in the udder and to prevent new intramammary infections (IMI) during the dry period (Berry and Hillerton, 2002a; Bradley et al., 2011). Teat sealants have also been shown to effectively prevent new IMI during the dry period (Huxley et al., 2002; Bhutto et al., 2011), especially if they are used in combination with antibiotics (Godden et al., 2003; Berry and Hillerton, 2007; Bradley et al., 2011). In this survey, the majority of farms used antibiotic intramammary tubes as a part of the dry-off procedure in combination with internal teat sealants.

It has been reported that high milk yield at dry-off increases the likelihood of milk leakage (Bertulat et al., 2013) and risk of new IMI (Dingwell et al., 2002; Rajala-Schultz et al., 2005;

Newman et al., 2010). Dingwell et al. (2001) pointed out that the application of antibiotics only at dry-off cannot completely prevent new infections occurring, highlighting the importance of reducing milk yield at dry-off.

Guidelines for dry-off procedures in the UK by AHDB Dairy encourage an abrupt dry-off when cows are producing less than 15 kg/day of milk. However, the current survey revealed that more than a quarter of cows still produced >20kg/day of milk at dry-off, and most of these cows were abruptly dried-off. High yielding cows (>20 kg/day) at dry-off showed higher udder pressure and higher cortisol level than low yielding cows (15< kg/day) at dry-off (Bertulat et al., 2013), suggesting that cows with high milk production at dry-off experienced discomfort in the udder. In the current survey, the level of milk production at dry-off was not associated with herd size, but was positively correlated with annual milk sales and average annual production level per cows. Therefore, high yielding cows and cows from farms with higher annual milk sales were potentially more likely to suffer from udder discomfort at dry-off.

It has been reported that intermittent milking successfully reduced milk production at dry-off (Green et al., 2007; Tucker et al., 2009; Newman et al., 2010; Zobel et al., 2013) and reduced the frequency of milk leakage after dry-off (Zobel et al., 2013). Therefore, it has been proposed as an alternative approach to abrupt dry-off. However, the current survey found that intermittent milking was not commonly practised. A reduction in milk production can also be achieved by feed restriction (Odensten et al., 2005, 2007b; Tucker et al., 2009), but a sudden reduction in feed quantity or quality has been shown to cause distress, frustration and hunger (Odensten et al., 2005; Valizaheh et al., 2008; Tucker et al., 2009). Compared to intermittent milking, feed restriction around dry-off was more commonly performed by respondents. Most methods of feed restriction involved reducing or removing concentrates, or reducing the quantity of a total mixed ration (TMR) being fed.

2.4.4. Feeding management

It was clearly shown from this survey that feed restriction was commonly performed after dry-off, with the lower feed quality for far-off dry cows evident in a reduction in silage, cereals and concentrate feeding. Feed restriction in the dry period has been a recommended procedure, since overfeeding dry cows can cause serious health problems after parturition (Agenäs et al., 2003; Holtenius et al., 2003; Douglas et al., 2006). Dann et al. (2006) found that feed restriction during the far-off dry period resulted in higher DMI and better energy balance after parturition. However, a sudden reduction in feed quantity or quality can be a major welfare concern. Behavioural changes associated with distress, frustration and hunger have been reported in cows as a result of feed restriction (Odensten et al., 2005; Valizaheh et al., 2008; Tucker et al., 2009). To avoid overfeeding whilst minimising hunger, some studies suggest adding chopped hay or straw into a mixed ration to create high-forage/low-energy diets (Beever, 2006; Janovick and Drackley, 2010; Vickers et al., 2013). Feeding high forage diets to dry cows seems to be widely implemented, as it can be seen that more respondent farms fed straw and hay for dry cows than for late lactating cows.

The majority of farms in this survey provided concentrate or non-forage feedstuff mixed with forage as part of the dry cow diet, and only a few farms fed concentrates separately. Feeding a TMR is a strategy to provide a homogeneous diet to all cows. In contrast, separate feeding of concentrates can lead to a rapid consumption of a high proportion of concentrate, which has been associated with an increased risk of metabolic disorders (Østergaard and Gröhn, 2000; Beauchemin et al., 2002; Maekawa et al., 2002). However, selective feeding has been reported in cows fed a TMR: cows normally select for concentrates and select against long fibrous feedstuffs (Leonardi and Armentano, 2003; DeVries et al., 2007). This could be problematic in a competitive situation, where subordinate cows can access the feed only after dominant cows have selected feed first (Hosseinkhani et al., 2008). Almost 90% of respondents answered that all dry cows have concurrent feed access. However, competition at the feed-face can occur even when all cows were able to feed at the same time (Lobeck-Luchterhand et al., 2015), and when the availability of feed was temporarily restricted (Collings et al., 2011).

Frequent feed delivery can shorten the time when a feed bunk is empty and ensure that the same quality of feed is always available (DeVries et al., 2005). This would allow cows to distribute their feeding time throughout the day, resulting in less competition at the feed bunk (DeVries et al., 2005; Collings et al., 2011). The delivery of fresh feed has also been reported to stimulate cows' motivation to feed (DeVries and von Keyserlingk, 2005). Most of the respondents delivered fresh feed for dry cows once a day, and only a few farms delivered twice a day. However, quite a few farms delivered fresh feed every second day or less, suggesting that cows in these farms may not have access to feed all the time. Together with constant access to the feed bunk, feed bunk management is an important factor to ensure that all dry cows consume an appropriate quantity of same quality feed. This would satisfy the energy requirements of cows for maintenance as well as those of the growing fetuses.

2.4.5. Housing systems and environment

Another recent UK survey (March et al., 2014) found that a summer grazing system for lactating cows (all day or part day) was commonly used. The current survey also found that cows in their late lactation and far-off dry periods were most commonly kept on pasture or outside in paddocks during the summer, and inside during the winter. A grazing system may be more beneficial to cows compared to continuous housing systems, allowing more natural behaviour while potentially reducing the risks of hoof damage (Somers et al., 2003; Haskell et al., 2006; Hernandez-Mendo et al., 2007; Burow et al., 2013) and mastitis (Green et al., 2007). Additionally, cows show a preference for spending time on pasture if they are allowed free access (Charlton et al., 2011).

However, full housing enables greater control over nutritional management, control of hypocalcaemia, and supervision at calving. The majority of farms kept their close-up dry and calving cows inside irrespective of the season of calving, probably because those cows in the transition period require more careful management compared to late lactation and far-off dry cows. The most common type of housing for close-up dry and calving cows was straw/sand yards, while there were also a small number of farms that used cubicles for their close-up dry cows and for calving. It has been reported that housing cows in cubicles during the last four weeks before calving extended the length of labour, and cows tended to take longer to stand up after calving, compared to cows housed in straw yards in the same period (Campler et al., 2015).

Green et al. (2007) reported that the hygiene level of dry cow cubicles and straw yards was associated with risks of mastitis after parturition. According to a survey conducted on organic and non-organic farms in the UK (Langford et al., 2009), passageways and lying areas for dry cows were generally not clean in either organic and non-organic farms. Therefore, if dry cows are kept in inside housing systems, it is important to keep the building clean in order to maintain good udder health of dry cows.

The Red Tractor Assurance for Farms - Dairy Scheme Standards designates 5.75 m^2 per cow as the minimum space allowance for cows kept in straw or sand yards, and at least one cubicle per cow for cows kept in a cubicle housing system. Overstocking of dry cows is not recommended because of its negative impacts such as decreased lying time and altered feeding behaviour (Lobeck-Luchterhand et al., 2015), and is counter to prepartum feeding management that aims to optimise feed intake. In the current survey, the majority of respondents thought that the photograph depicting 6.7 m^2 per cow was the most appropriate, and almost half of the respondents thought that the appropriate number of cows in a shed with 100 cubicle beds was 90 cows. It would appear that respondent farmers have a good appreciation of the importance of avoiding over-stocking in late pregnancy.

2.4.6. Calf management

The majority of respondent farms separated calves from dams within 24 hours, with 29% of them separating at less than six hours after birth. The main reason for an early separation in dairy farms is to maximise productivity in cows and to control infectious diseases in calves. Lack of maternal care in early life has been shown to have a negative influence on the health, growth and behaviour of calves (Weary and Chua, 2000; Flower and Weary, 2003; Stěhulová et al., 2008). The current survey found that smaller farms tended to keep calves with dams longer than one day, but the majority of larger farms separated them immediately after birth.

After separation from the dam, calves on respondent farms were kept in either individual hutches/crates or group housing. Less than a quarter of respondent farms solely used an individual housing system for their pre-weaned calves. The benefit of an individual housing system is that it allows for the easy monitoring of milk intake and calf health. However, some drawbacks of individual housing have been reported e.g. higher reactivity to novel environments and unfamiliar calves (Jensen et al., 1997; de Paula Vieira et al., 2012b), impaired cognitive ability (Gaillard et al., 2014), and lower social rank in group environments later in life (Veissier et al., 1994). A larger space allowance in group housing can provide calves with the opportunity to exercise, which is often restricted in individually housed calves (Jensen, 1999). In this survey, a combination of individual and group housing systems was most commonly used.

Although group housing of pre-weaned calves has become increasingly common (Hepola, 2003), little information is available regarding the optimum time to introduce calves into a group environment. In this survey, the timing of introduction to group housing varied from soon after birth to 40 days of age. It has been reported that a calf's social motivation markedly increases from two to three weeks of age (Vitale et al., 1986; Sato et al., 1987; Duve and Jensen, 2012). In the group environment with an automatic milk feeding system, calves introduced at six days old had a more difficult time adapting to a new system and integrating into a group than calves introduced at an older age (Rasmussen et al., 2006; Jensen, 2007; Fujiwara et al., 2014). This survey did not ask if farms used an automatic feeder in their group housing, but the majority of farms moved their calves to group housing within two weeks of age. Although the main purpose of using group housing systems is to reduce labour and to provide calves with a more natural environment, younger calves might not benefit from this system as much as older calves.

Calf group size also showed a large variation. There is limited information regarding the optimal group size for pre-weaned calves, but Færevik et al. (2007) reported that social conflict does not necessarily derive from larger group sizes. On the other hand, a larger group size might be problematic if resources were limited e.g. if there is only a single milk feeding station for a large group of calves. However, the outweighing benefit of social experience in early life has been well documented (e.g. de Paula Vieira et al., 2012; Jensen et al., 1997).

The current survey found that more than half of respondent farms gave four litres or less of milk per day for pre-weaned calves, which was a much smaller amount than AHDB Dairy recommendations (13-15% of calf birth weight per day i.e. 5-6 L/day). Restricted milk feeding for pre-weaned calves is common, probably due to a common belief held by dairy farmers that feeding higher amounts of milk can cause diarrhoea (AHDB Dairy, 2010). However, in order to optimise future profitability, it is critical for farmers to offer adequate nutrition to pre-weaned calves (Appleby et al., 2001; Khan et al., 2007). Relatively recent surveys on calf management in other countries reported that producers normally gave six litres (median) of milk per day for calves a week before weaning (Czech Republic: Staněk et al., 2014; Canada: Vasseur et al., 2010). Numerous studies have shown that calves are capable of ingesting more than six litres of milk per day, and that increased milk intake enhances their growth during the pre-weaning period (Appleby et al., 2001; Jasper and Weary, 2002; de Paula Vieira et al., 2008; Borderas et al., 2009b; de Passillé et al., 2011; Miller-Cushon et al., 2013). Moreover, a more recent study has shown that there is a positive relationship between pre-weaning growth and milk production in the first lactation (Soberon et al., 2012). Knowledge exchange in this field might, therefore, be of great benefit to farmers in terms of maximising cow lifetime productivity.

Calves on respondent farms were weaned off milk at a median of eight weeks of age, but there was a large variation. In Europe, EU Council Directive 2008/119/EC prohibits keeping calves aged more than eight weeks in individual pens. Therefore, it is likely that calves reared in an individual housing system during the entire pre-weaning period are weaned at eight weeks of age i.e. when they join group housing. Weaning normally took place over two to seven days, but 19% of respondent farms abruptly stopped feeding milk at weaning. Abrupt weaning has been reported to increase vocalisation (Budzynska and Weary, 2008), incidence of cross-sucking (Nielsen et al., 2008) and the frequency of visits to a milk feeder (Nielsen et al., 2008), which indicates hunger and poor welfare.

Disbudding and dehorning are common but painful procedures that most heifer calves encounter in their early life (Stafford and Mellor, 2011; Wikman et al., 2013). It is widely practised for safety reasons for both animals and handlers. In the UK, it is compulsory to use local anaesthesia when calves are disbudded, except for chemical cauterisation. Calves experience pain during disbudding regardless of the method used (Stafford and Mellor, 2011), but Vickers et al. (2005) suggested that chemical burning by caustic paste might be less painful than cautery disbudding. From this survey, the majority of farmers used hot iron dehorning with local anaesthesia, and only four farms used caustic paste. Surprisingly, two farms admitted that they did not use local anaesthesia. The application of local anaesthesia has been reported to alleviate pain for at least a couple of hours after the procedure (Morisse et al., 1995; Graf and Senn, 1999; Grøndahl-Nielsen et al., 1999), but Stafford and Mellor (2005) argued that local anaesthesia cannot prevent pain associated with inflammation which lasts for a couple of hours after the procedure. Administration of analgesics has been reported to alleviate pain after the effect of local anaesthesia wore off (Stewart et al., 2009; Sutherland et al., 2002). Unfortunately, the use of analgesics was not queried in this survey, but such a question would have enabled us to see how many farmers perceived disbudding as a painful procedure for young stock.

2.4.7. Farmer's perception of the three most important periods in dairy management

The majority of respondents chose the early lactation period and the close-up dry period as the most important period in the production cycle of dairy cows. Cow health in the early lactation period directly reflects the perception that it is primarily the lactating cows that provide the income for the farm, and it is this period when milk production of cows is at its highest. The close-up dry period is well known to affect the health and the productivity of cows after parturition, which also directly affects profitability. Young-stock seems to be the third most important period for the majority of farmers, suggesting that farmers have an understanding of the importance of rearing healthy replacement heifers to ensure future profit.

2.5. Conclusions

These survey results provide an insight into the typical experiences of late gestation cows on large commercial farms in the UK, but a significant herd size association was only found with the maximum group size for dry cows and the timing of separation of calves from dams. Some of the welfare recommendations given in the literature do not appear to be fully implemented. For example, an abrupt dry-off of high yielding cows would not only cause udder discomfort but would also reduce the effectiveness of dry cow therapy. The development of a suitable method to reduce milk production at dry-off is one area that needs to be addressed. The current survey showed that farmers had a good appreciation of industry recommendations for stocking density. As expected, the majority of farms had dynamic social groups for dry cows. This may result in social stress that can, directly and indirectly, affect disease susceptibility and so efforts to maintain a stable social environment for dry cows should be emphasised. Moreover, although farmers understand the importance of replacement heifer management for their future profit, the responses collected in the calf management section may suggest that some management practices associated with compromised calf health and welfare were commonly used. The current survey provides useful information for dairy farmers and scientists to identify potential sources of stress associated with common dry cow management and pre-weaned calf management practices, and to implement methods that can reduce the negative impacts on the health and production of cows and the future performance of calves.

Chapter 3 :

Effects of stocking density during the dry period on dairy cow activity, physiology, metabolism and behaviour

3.1. Introduction

Prepartum health and welfare in dairy cows is of particular importance as it can influence performance after calving. It has been reported that a decline in prepartum feed intake results in peripartum negative energy balance, which is associated with increased risks of metabolic disorders and production loss (Grummer, 1995; Rabelo et al., 2003; Huzzey et al., 2007). In the late gestation period, cows normally go through changes in physiology, metabolism and the immune system. Additionally, management practices for dairy cows in the last one or two months of the gestation period (dry period) are very different from the lactating period. This includes cessation of milking and a change of diet. Moreover, the survey conducted on UK dairy farms has shown that dry cows are often kept in dynamic social groups, and an individual cow may move between management groups in the middle of the dry period, according to the stage of gestation (e.g. far-off to close-up dry group: see **Chapter 2**). This is sometimes accompanied by a change in housing systems (e.g. pasture/cubicle shed to straw yard: see **Chapter 2**).

Group housing is beneficial for dairy cows, as it enhances feed intake and natural behaviour (Albright, 1993). However, group environments can be stressful if animals have to compete for scarce resources such as feed and lying areas. Regrouping of unfamiliar animals has also been shown to increase agonistic social interactions (e.g. pigs: Jarvis et al., 2006; cows: von Keyserlingk et al., 2008). This suggests that dynamic social grouping (i.e. the continual entry of new cows and removal of cows to other groups) can be a potential source of social stress for dry cows. Proudfoot and Habing (2015) suggested that there may be a linkage between social stress and diseases in farm animals, and outlined some experimental and observational evidence that some management practices can play a key role in exposing farm animals to social stress.

Overstocking of dairy cows is common especially on large farms, as it can maximise profit while reducing cost (Estevez et al., 2007). Rioja-Lang et al. (2012) suggested that the minimum feed bunk space per cow should be at least 0.6 m to ensure subordinate cows have access to feed, but studies on North American farms found that some farms provided less than 0.6m of feed bunk space per cow (Endres and Espejo, 2010; King et al., 2016). Charlton et al. (2014) found that the lying cubicle stocking density in Canadian dairy farms varied from 52% to 160%, and cows on the farms with more than 100% stocking density did not reach a target daily lying time (12h/d). The UK Code of Recommendations for the Welfare of Cattle (DEFRA, 2003) does not specify the exact space allowance for feeding and lying space for dairy cows, and recommends providing cows with enough feeding space and 5%

more cubicles than the number of cows in a group. It is uncertain how many of these recommendations are implemented on UK dairy farms, but it is possible that cows on large farms experience competition at the feeding and lying areas due to limited space allowance.

The negative effects of overstocking have been well documented in dairy cows, and include altered feeding behaviour, increased competition at the feed-face and decreased lying time (Huzzey et al., 2006; Fregonesi et al., 2007a; Krawczel et al., 2008; Proudfoot et al., 2009; Collings et al., 2011). Moreover, overstocking of transition cows could induce physiological stress responses, evident in an increase in circulating stress-related hormones (Fustini et al., 2017) or faecal glucocorticoid metabolites (Huzzey et al., 2012). It has been reported that frequent regrouping of prepartum cows increases competition at the feed-face (Schirmann et al., 2011; Lobeck-Luchterhand et al., 2014). Moreover, Talebi et al. (2014) found that a reduced stocking density resulted in reduced competition at the feed-face and extended lying times after regrouping. During the dry period, cows could be more subject to risks of overstocking since their social structure is regularly changing. Therefore, it is important to ensure adequate space for prepartum dairy cows.

Previous studies on stocking density in prepartum dairy cows have used different experimental settings. For example, Huzzey et al. (2012) compared 0.67m feed bunk space with 1.0 cubicle per cow, and 0.34m feed bunk space with 0.5 cubicles per cow. Fustini et al. (2017) compared 12.0m^2 and 4.8m^2 space allowance per cow. Studies on Jersey cows used 80% stocking density for both feeding and lying space in comparison to 100% (Silva et al., 2014, 2016; Lobeck-Luchterhand et al., 2015). All of these studies showed that increasing stocking density resulted in more competition at the feed-face. However, these studies were conducted over a relatively short period (14 days: Huzzey et al., 2012; 21 days: Fustini et al., 2017; four weeks: Lobeck-Luchterhand et al., 2015), with a relatively small group size (ten cows per group: Huzzey et al. 2012; two and five cows per pen: Fustini et al. 2017).
Therefore, the aim of this experiment was to investigate the effect of overstocking during the entire dry period in more industry-relevant settings. The current study hypothesised that high stocking density during the dry period would result in more frequent agonistic social interactions, altered feeding and lying behaviour, the activation of physiological stress responses and negative energy balance in the dry cows, and that there would be carry-over effects on health and productivity during the subsequent lactation period. To examine the effect of maternal exposure to high stocking density during the dry period on calf performance, calves being carried by these cows were followed until weaning in the subsequent study (see **Chapter 4**).

3.2. Materials and methods

3.2.1. Animals and housing

The experiment was conducted between 26th November 2014 and 4th July 2015 at the SRUC Dairy Research and Innovation Centre (Dumfries, UK). The experiment was approved by the SRUC Animal Welfare and Ethical Review Body (Animal Experiment Number: AE 41-2014). Forty-eight prepartum Holstein Friesian cows (parity ≥1) were enrolled in this study immediately after dry-off and were returned to the lactating herd after calving. The cows were dried off 60 (±4) days before their expected calving date and kept in a cubicle pen until 21±4 days before the expected calving date (far-off dry group), and then moved to a straw yard until the first milking after parturition (close-up dry group). Dry-offs and movements of cows from cubicle pens to straw yards occurred on Wednesdays at approximately 14:30 between 26th November 2014 and 29th April 2015, and on Wednesday mornings at approximately 09:30 between 6th May and 2nd July 2015. Occasionally, cows were dried-off and moved on Tuesdays for management reasons.

The layout of the building and experimental pens are shown in **Figure 3.1A**, **B**, and **C**. Cubicle pens for dry cows were located on both sides of the building, adjacent to lactating cow groups. Straw yards were located at the back of the building, adjacent to the milking parlour and the collection yard. Experimental pens were of a symmetrical design for each of the treatment groups in both the cubicle pens and the straw yards. Each cubicle (2.60m length × 1.15m width) was covered by a mattress and bedded with sawdust. New sawdust was added to the cubicles every day, and passageways were cleared using automatic scrapers throughout the day. The straw yards had a straw-bedded lying area and loafing area with a concrete floor. Once per week, dirty straw and manure in the loafing area were removed using a tractor-driven scraper, and new straw was added in the lying area. An individual yoke feed barrier (0.4m width/yoke) was used as the feed-face in the cubicle pens, and a post-and-rail feed barrier (4.8m width) was used in the straw yards. A fresh dry ration was delivered once per day between 11:00 and 16:00 for both far-off and close-up dry groups, and feed was pushed up once per day between 17:00 and 19:00. Cows had *ad libitum* access to water *via* a water trough.

3.2.2. Treatment

Subject cows were randomly allocated into either high (H) or low (L) stocking density groups immediately after dry-off (H: n=25, L: n=23). The treatment groups were balanced for parity. The H group had 0.5 feed yokes and 1.0 cubicle per cow during the far-off dry period, and 0.3m of linear feed-face space and 6.0m^2 of lying space per cow during the close-up dry period. The L group had 1.0 feed yoke and 1.5 cubicles per cow during the far-off dry period, and at least 0.6m of linear feed-face space and at least 12.0m^2 of lying space per cow during the close-up dry period (**Figure 3. 2**). In the cubicle pens, gates blocked passageways in the feeding and cubicle areas to separate dry cows from lactating cow groups. In straw yards, gates enclosed dry cows within an appropriate space for each of the treatment groups.

Group composition changed every week due to the addition of new dry cows to the far-off groups and the movement of far-off cows into the close-up groups. Group size in the close-up groups also changed after the removal of calved cows. The gates were therefore re-adjusted every time the group size changed in order to maintain the same stocking density. Wood panels were used to block the feed-face in the close-up H group to limit access of cows to the feed-face. Since the minimum feed-face space allowance for L group was 0.6m/cow and the group size for close-up L group never exceeded eight, the feed-face for close-up L group was always left open at 4.8m. Due to limited space for non-lactating cows on the farm, both treatment groups included cows that were not used for the experiment (non-focal cows). Those cows were either expected to be culled, sold or dried-off far earlier than 60 days before the expected calving date. Gates and wood panels were re-adjusted after these cows left the groups to maintain appropriate experimental conditions.

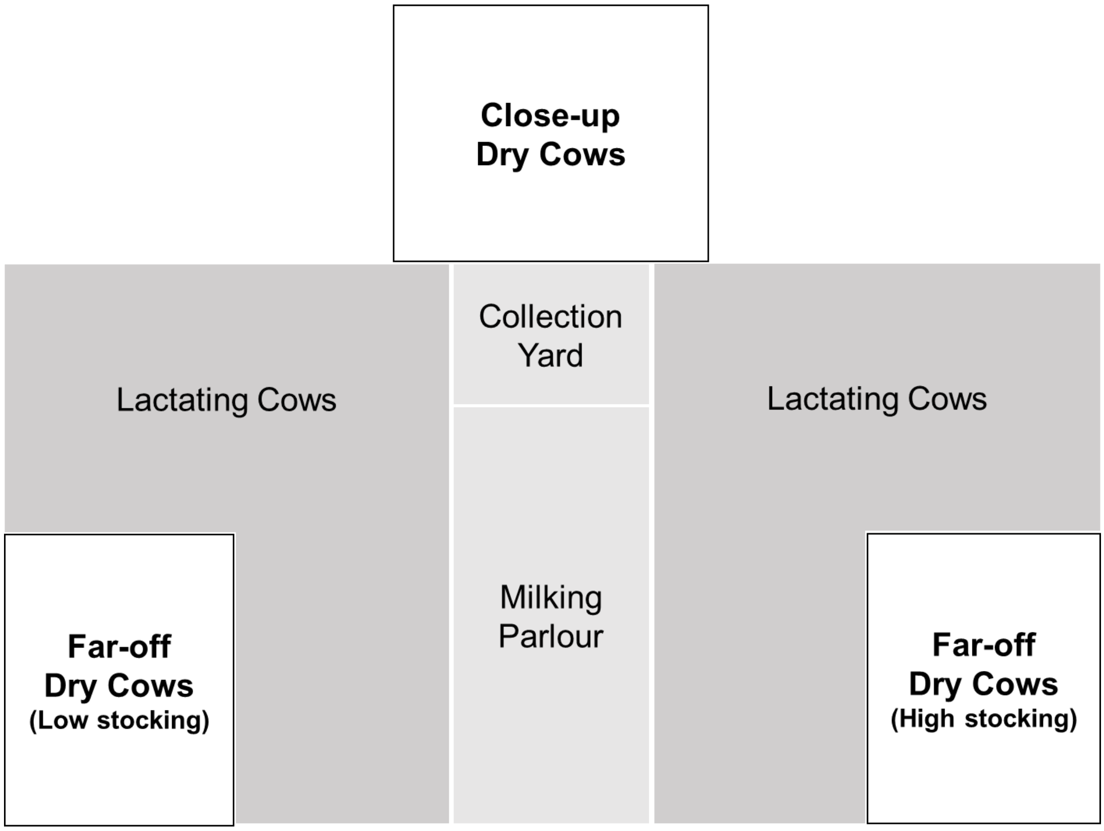

Figure 3.1A. Layout of the building for lactating and dry cows.

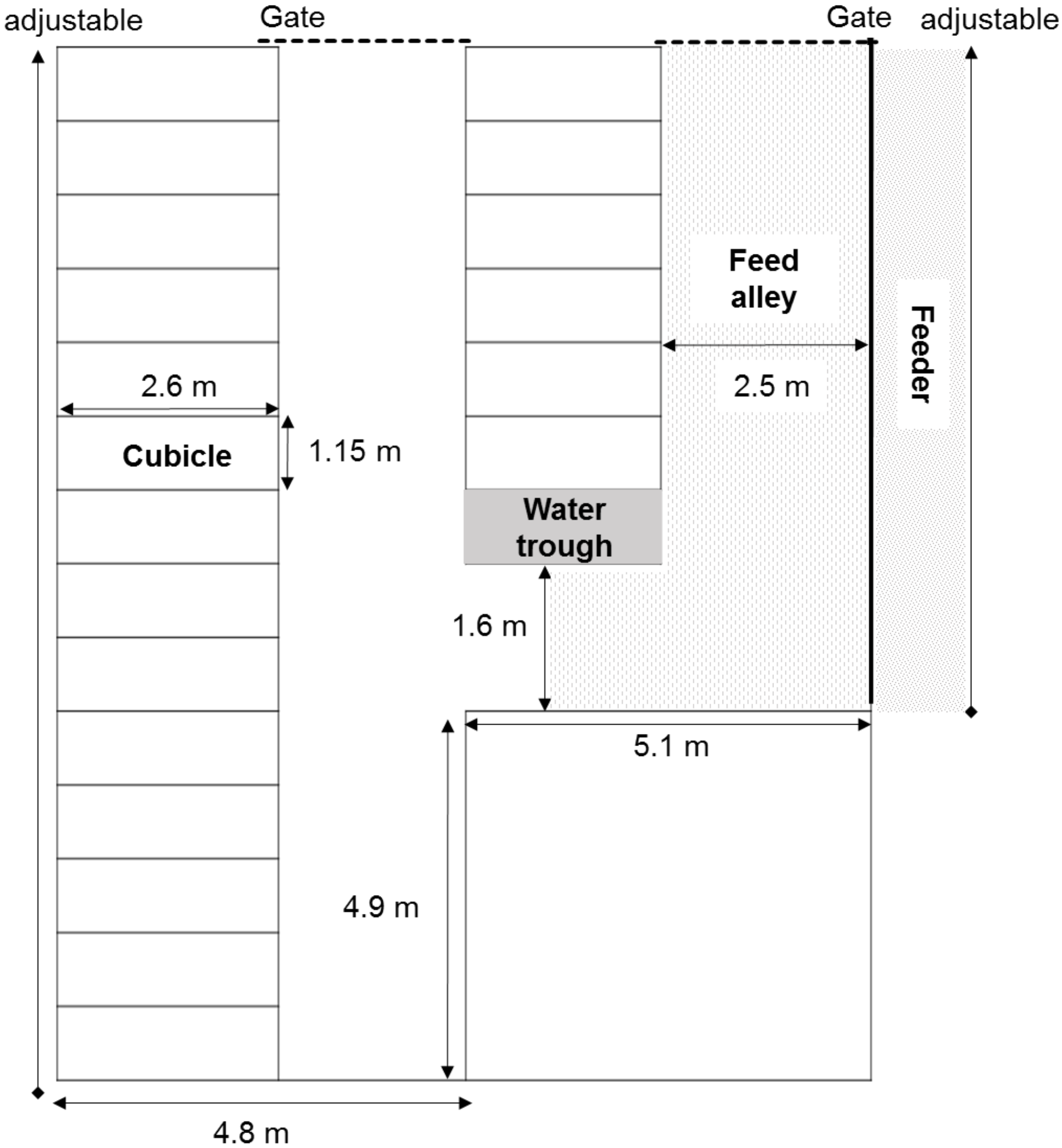

Figure 3.1B. Layout of the cubicle pen for L group (H group was a symmetrical design).

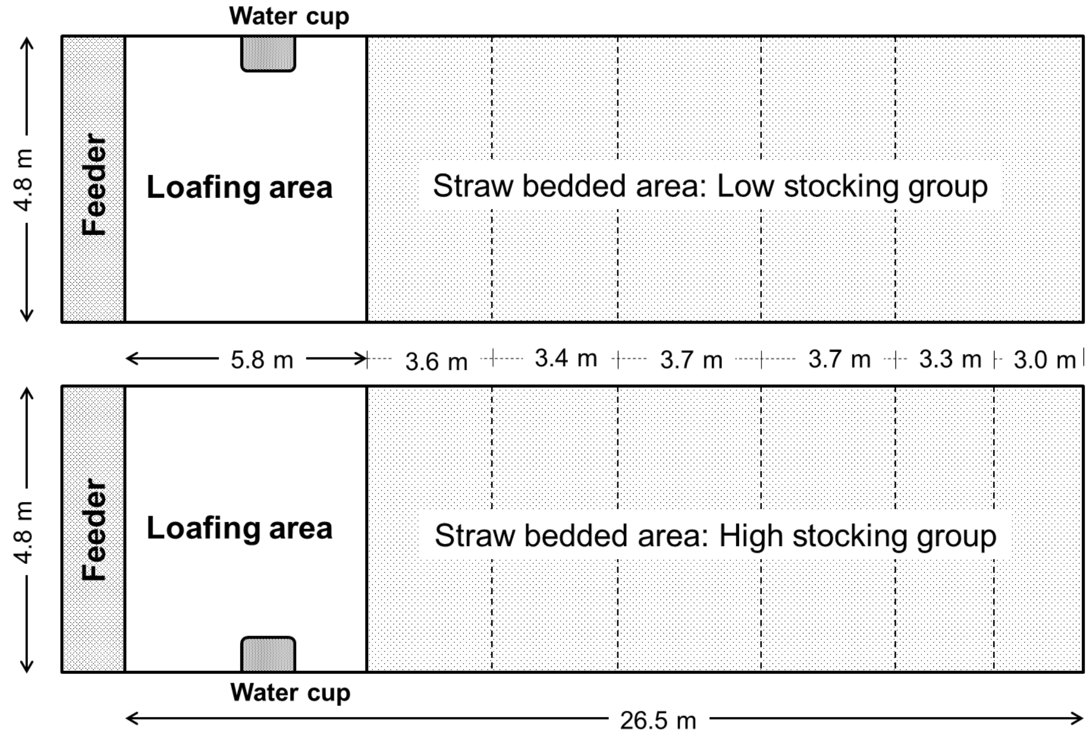

Figure 3.1C. Layout of the straw yards, dotted lines indicate the possible location of a gate to maintain experimental stocking density.

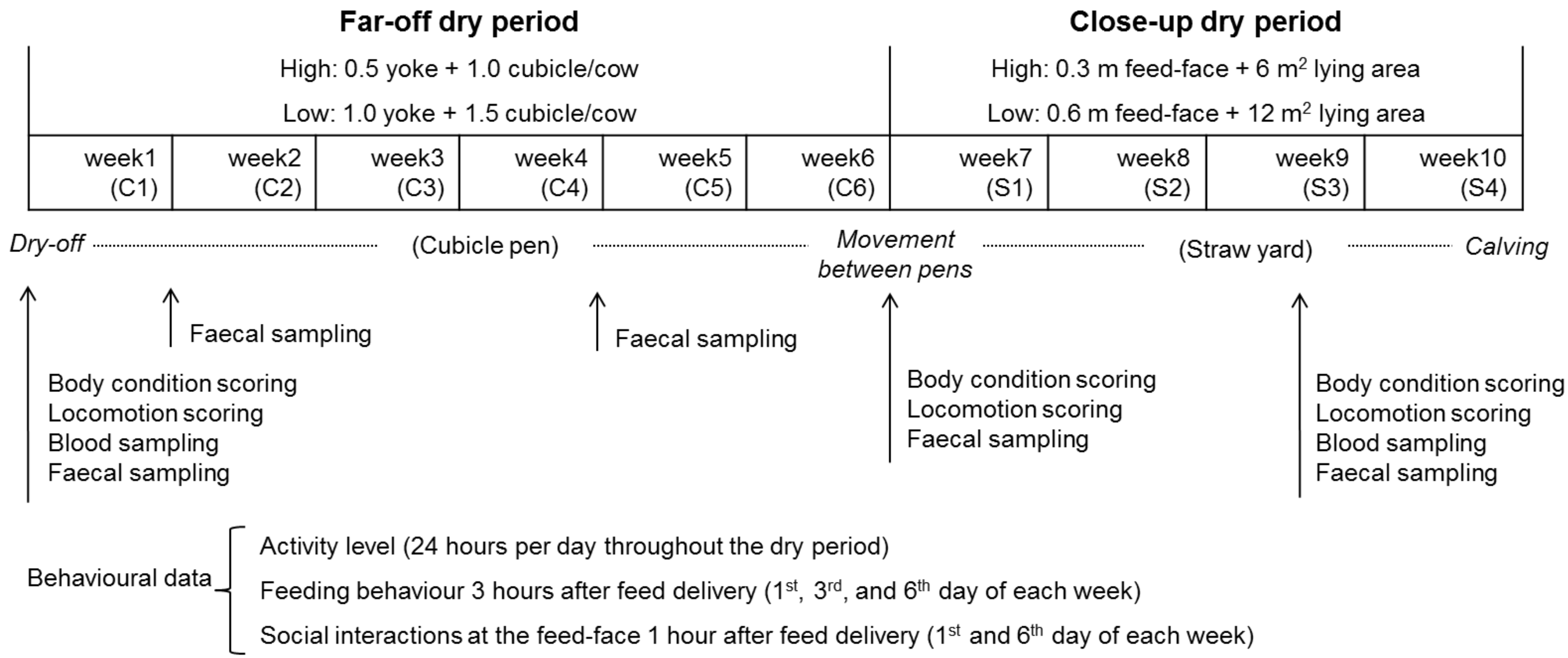

Figure 3. 2. Experimental setting and data collection points during the stocking density treatment period (from dry-off to calving). Cows were kept in a cubicle pen after dry-off until 21±4 days before the expected calving date (far-off dry period). Cows were moved to a straw yard until calving (close-up dry period). Bracketed variables (C1-C6 and S1-S4) indicate weeks in the cubicle pen and weeks in the straw yard. Arrows indicate the timing of body condition scoring, locomotion scoring, and physiological data collection. Behavioural data were collected throughout the dry period.

3.2.3. Data collection

3.2.3.1. General information

Daily milk yield on the day before dry-off (MY dry-off) and the average daily milk yield from 5 to 30 days after parturition (MY first month) were obtained from the herd management program (DairyPlan Herd Management Software, GEA Farm Technologies, Cheshire, UK). Data for 305d milk yield during the previous lactating period (305d MY), somatic cell counts from the latest recording before dry-off (SCC dry-off) and the first recording after calving (SCC first month) were downloaded as a CDL file from The Cattle Information Service and imported into herd management software (InterHerd; National Milk Records, Chippenham, UK).

The timing of data collection for each cow during the treatment period is illustrated in **Figure 3. 2**. Body condition score (BCS) and locomotion score (LS) were measured on three occasions: at dry-off, when cows were moved to the close-up dry group (transition) and 7±5 days before the expected calving date (pre-calving). BCS was measured using a 0-5 scale with 0.5 intervals (Mulvany, 1977 modified) and LS using a 1-5 scale (1=sound, 5=severely lame: Manson and Leaver, 1988).The first milk after parturition (colostrum) was collected in the parlour, and the colostrum quality was measured using a colostrum densimeter (KRUUSE colostrum densimeter, KRUUSE, Langeskov, Denmark). The concentrations of colostrum Ig (mg/ml) were corrected at 20 ˚C using the formula by Mechor et al. (1991).

The incidence of mastitis, metabolic disorders and other infectious diseases during the dry period (disease incidence dry period) and the first month of the subsequent lactation period (disease incidence first month) were recorded as per standard farm practice.

3.2.3.2. Physiological data

Faecal samples were collected at five different sampling points: at dry-off, on the seventh day (week2) and 35th day (week5) after dry-off, at the movement of a cow from far-off to close-up group (transition) and 7±5 days before the expected calving date (pre-calving). Samples collected were sealed in plastic bags, homogenised and stored at –20 ºC. Steroids from the faecal samples were extracted by mixing each of the raw faecal samples (0.50±0.01g) with 80% methanol (5.0ml). Mixed solutions were centrifuged for 20 minutes at 2,500×*g* and the supernatant was collected and stored at –20°C until analysis (Palme and Möstl, 1997). Concentrations of faecal glucocorticoid metabolites (11,17-di-oxoandrostane; 11,17-DOA) were measured using a competitive enzyme immunoassay (Palme and Möstl, 1997, modified for use in cattle Palme et al., 1999). Intra-assay Coefficient of Variability

(CV) of samples and inter-assay CV calculated for the 11,17-DOA assays were <13.0% and 4.2% respectively.

At dry-off and pre-calving (7±5 days before the expected calving date), blood was collected from the coccygeal vessel into 10-mL sterile plain tubes and 10 mL sterile tubes contained sodium fluoride. After centrifugation (3,000×*g* for 10 minutes), serum and plasma were collected and stored at –20 ºC until analysis. Concentrations of serum nonesterified fatty acids (NEFA), β-hydroxybutyric acid (BHB) and plasma glucose (Glu) were measured using an Instrumentation Laboratory IL600 wet chemistry system using reagents supplied by Randox (BHB), Alpha Laboratories (NEFA) and Instrumentation Laboratory (Glu) by the Dairy Herd Health and Productivity Service at the Royal (Dick) School of Veterinary Studies, University of Edinburgh.

3.2.3.3. Activity monitoring

At dry-off, all cows were fitted with a triaxial accelerometer (IceTag Pro: IceRobotics Ltd, South Queensferry, Edinburgh, UK) on a hind leg to monitor activity levels throughout the experimental period. The IceTag Pro collects orientations of the device on the x, y and z place at a rate of 16 times per second, which is interpreted to derive lying and standing times (MacKay et al., 2012). IceTag Pro also calculates activity as MotionIndex (a measure of acceleration summed over each plane over a given period) which then estimates the step count (MacKay et al., 2013). Data were downloaded using IceManager (IceRobotics Ltd, South Queensferry, UK) and converted to comma-separated values (CSV) files, which provided MotionIndex (MI), step count (SC), lying and standing duration per minute. The true frequency of lying bouts (LB) and true lying and standing durations were calculated using formulae that eliminate false lying bouts (any lying bout lasting less than four minutes; see Tolkamp et al., 2010). Daily MI, SC and LB were obtained from the data, and daily lying proportion (LP) was calculated by dividing lying duration by summation of daily lying and standing durations.

3.2.3.4. Behavioural observations

Behaviour at the feeding area (feed-face, feed alley, loafing area) was continuously monitored from 10:00 to 19:00 with waterproof infrared CCTV cameras (1/3’’ Sony Color CCD, EZ420IR-30, ezCCTV.com Ltd, Herts, UK) connected to a digital video surveillance system (GeoVison, version8, GeoVision Inc., Taipei, Taiwan). Three observation days per week were selected (one day after weekly regrouping called observation A; three days after weekly regrouping called observation B; one day before weekly regrouping called observation C). The video footage for these days was saved onto hard disks for later observation. Focal cows were marked with individually allocated numbers on both sides of their rumps using hair dye. All of the video footage was observed by a single observer for the entire experimental period (32 weeks). There were days when the video footage was not stored in the computer, and these days were treated as missing data. For each of the behavioural observations described below, intra-observer reliability was assessed before the start of the actual observation, by watching the same video clips for three different days twice over.

Feeding behaviour

Video observation started when fresh feed was delivered to the feed bunk. Cows' feeding behaviour was observed on three observation days (A,B,C) by continuous and scan sampling. Continuous observation started at the time of feed delivery (between 11:00 and 16:00). The times when each cow was first seen standing in the feed alley and when she started feeding were recorded. A cow was recorded as "standing in the feed alley" when its shoulder was within the feed alley or loafing area including water trough, and the cow was not feeding. A cow was recorded as "feeding" when its head completely crossed the line between the feed alley and the feed bunk. Latency to approach the feeding area and latency to start feeding were calculated by subtracting the time of feed delivery from the time when a cow was first observed standing in the feed alley and the time when a cow started feeding, respectively. Information from cows that did not appear at the feeding area while the video was recording (from the time of feed delivery to 19:00) was treated as censored data.

Five-minute scan sampling was used to record if a cow was standing in the feed alley and if a cow was feeding in the first three hours (3h) after feed delivery. The total number of times a cow was either standing in the feed alley or a cow was feeding in the 3h after feed delivery (H0-3) was obtained for each observation day. The total number of times a cow was feeding in 0-1h (H0-1), 0-2h (H0-2), 1-2h (H1-2) and 2-3h (H2-3) after feed delivery were also calculated for each of the observation days.

Social interactions at feeding area

Agonistic and non-agonistic social interactions at the feed-face were continuously observed for three different periods (0-20min, 40-60min, 80-100min) after feed delivery for two days per week (observation A and C). Behaviours observed during the three time periods were summed to obtain values per observation day. Physical (push, butt, block) and non-physical (threat) aggressive actions and resilient response (no response, push back) and passive response (avoids, half displaced, displaced) to the actions were recorded (**Table 3.1**), as well as the identities of both actor and recipient cows. Non-focal cows were recorded as cow X with no individual distinction.

A "voluntary leave" was recorded if a cow left the feeding area within two minutes after being displaced to assess how many cows stopped feeding after losing access to the feed-face because of displacement (**Table 3.1**). Non-agonistic social interactions included non-aggressive physical contacts with a neighbour cow, which resulted in a focal cow being displaced or half displaced from the feed-face (**Table 3.1**).

Table 3.1. Ethogram for aggressive and non-aggressive interactions at the feed-face.

Aggressive interactions	**Description**
Actor	
Push/Butt	The cow applies forces to the recipient using its head or some other part of its body to displace the recipient
Block	The cow stands still and uses its body to block the recipient gaining access to the feed-face
Threat	The cow presents a threat posture by presenting its forehead with inclined head or the cow engages in a threatening swing of the head in the direction of the recipient but no contact occurs between the two individuals
Recipient	
No response	The cow shows no physical response
Push back	The cow pushes the aggressor back so that the aggressor does not displace the recipient
Avoid	The cow moves/turns its head away in the opposite direction from the actor
Withdraw side/back (=Half displace)	The cow withdraws its head from the feeder after the aggressive interaction (push/butt/block) and/or moves along to the right/left, or moves back less than one cow body-length from the feed-face
Displace	The cow withdraws its head from the feeder after the aggressive interactions and moves backwards until her shoulder line is beyond one cow body-length
Voluntary leave	The cow moves away from the feeding area within 2 minutes after being displaced
Non-aggressive interactions	**Description**
Behaviour	
Withdraw side/back (=Half displaced) without aggressor	The cow withdraws its head from the feeder and/or moves along to the right/left, or moves back less than one cow body-length caused by non-aggressive physical contact
Displaced without aggressor	The cow withdraws its head from the feeder and moves backwards until her shoulder line is beyond one cow body-length as a result of a non-aggressive physical contact

3.2.4. Data analysis

Statistical analyses were performed using Genstat® 16th Edition (VSN International Ltd, Hemel Hampstead, UK) and figures generated using Excel 2013. Data were first tested for normality and transformed where necessary. LS (1-5 scale) was categorised as lameness score (lame: LS≥3, non-lame: LS≤2), and parity (1-7) was categorised as either primiparous (P: parity at dry-off=1) or multiparous (M: parity at dry-off ≥2). Three cows were treated with tylosin antibiotic (Tylan® 200, Elanco Animal Health, IN, USA) during the dry period (H: n=1, L: n=2) because of severe lameness. All the variables of interest during the dry period were first analysed with or without these three cows. The inclusion of these three cows did not affect the results, so these three cows were included in all of the subsequent analyses. A post-hoc analysis (Fisher's unprotected least significant difference test) was conducted when there were significant interactions or significant differences between more than two categories (e.g. sampling points) to investigate the direction of the effect. Normality of the residuals was checked graphically.

3.2.4.1. Descriptive data of the herd

Summary statistics were calculated to test the normality of the data and a logit transformation was used for "SCC dry-off" and "SCC first month". Non-parametric tests were used when transformations did not normalise data. The two-sample T-test was used to analyse "305d MY" and "SCC dry-off", and the Mann-Whitney U test was used to analyse "MY dry-off" as data were not normalised by using a logit transformation. The number of cows receiving any veterinary treatment during the subsequent lactation period was analysed using a Chi-square test, as the number of animals treated for each of the reported disease variables was insufficient to conduct individual analyses for each disease.

The change in BCS during the dry period was analysed by linear mixed model using residual maximum likelihood procedures (REML) including treatment (H, L), time point (dry-off, transition, pre-calving) and the interaction between treatment and time point as fixed effects, and cow as a random effect. A change in lameness score (lame or non-lame) during the dry period was analysed with generalised linear mixed model (GLMM) using a binomial distribution with a logit function. The model included treatment, time point and the interaction between treatment and time point as fixed effects and cow as a random effect.

REML was used to analyse dry period length (DPL), gestation period length (GPL) and corrected colostrum Ig. The model included treatment and parity (M, P) as fixed effects and cow as a random effect. REML was also used to analyse "MY first month" and "SCC first month", with an initial model including treatment, parity and disease incidence in the first month as fixed effects. However, disease incidence was removed from the final model as it had no significant effect, and disease incidence was not the main interest of this study. The final model for "MY first month" fitted treatment and parity as fixed effects and cow as a random effect. The final model for "SCC first month" included "SCC at dry-off" as a fixed effect in addition to treatment and parity, and cow as a random effect.

3.2.4.2. Physiological data

The day at which pre-calving samples were taken varied from 1 day to 23 days relative to calving. The data from three cows sampled within 48 hours of calving were removed from the physiological data analyses because cows experience dramatic changes in physiology and metabolism around calving (Goff and Horst, 1997; Roche et al., 2013). The data from a cow sampled at -23 days relative to calving were also removed from the analysis for assessment of energy balance, as this was considered too early to properly evaluate pre-calving metabolic status. Levels of faecal glucocorticoid metabolites (FGCM) were analysed using REML following a logit transformation. Treatment, parity and sampling points (dry-off, week2, week5, transition, pre-calving) were included as fixed effects as these were of main interest for the study. Interactions between treatment and parity, BCS, LS and disease incidence during the dry period were dropped from the final model as they were not significant ($p>0.05$).

Metabolic parameters at dry-off (Glu1, NEFA1, BHB1) and pre-calving (Glu2, NEFA2, BHB2) were analysed separately by GLMM using a binomial distribution with a logit link function. Treatment and parity were fitted as fixed effects and cow as a random effect. BCS, LS and the interaction between treatment and parity were not included in the final model, as they were not significant. Residual plots obtained from the initial analyses for NEFA1, NEFA2 and BHB2 showed that these values required a logit transformation. The change in energy parameters between dry-off and pre-calving, obtained by subtracting values at dry-off from values at pre-calving (Δpre-dry: Glu2-1, NEFA2-1, BHB2-1), were analysed using GLMM. Initial analyses included changes in BCS and LS from dry-off to pre-calving as fixed effects in addition to treatment and parity, but the change in BCS was dropped from the final model as it was not significant. Although the change in LS showed a significant effect on BHB2-1 ($P=0.003$), the model could not accurately predict the means for multiparous and primiparous cows due to unbalanced data distribution (no multiparous cows had an increase in LS over the dry period). Therefore, LS was also dropped from the final model.

To investigate whether there was a treatment effect on the likelihood of developing postpartum disorders and milk loss, a Chi-square test was conducted to compare the percentages of cows that had NEFA2>0.50 nmol/L in each of the treatment groups. Fisher's exact test was conducted to compare the percentages of cows that had NEFA2>0.30 nmol/L or BHB2>0.60 nmol/L in each of the treatments. The higher concentrations of prepartum NEFA and BHB are associated with health, productive and reproductive performance during the subsequent lactation period (Ospina et al., 2010a; Chapinal et al., 2011, 2012). For example, cows showing pre-calving serum NEFA concentrations ≥0.50 nmol/L had an increased risk of experiencing a displaced abomasum after calving (LeBlanc et al., 2005; Chapinal et al., 2011), and those cows with NEFA concentration ≥0.30 nmol/L had a higher risk for other metabolic disorders such as retained placenta and metritis (Chapinal et al., 2011). Prepartum NEFA≥0.30 nmol/L and BHB≥0.60 nmol/L have been associated with reductions in postpartum milk production (Chapinal et al., 2012).

3.2.4.3. Activity levels of the cows

The activity levels of the cows from dry-off to two days before calving were used for the analysis. The data during the last 48 hours before calving were not included in the analyses in order to eliminate the effect of a known behavioural change immediately prior to calving (Huzzey et al., 2005; Kok et al., 2015). Effects of factors of interest on the activity levels of the cows (MI, SC, LB, LP) were analysed using REML. All of the variables except LP were logit transformed. The effect of changes over week from dry-off (1-10) and housing type (cubicle/yoke, straw/post-rail) were combined and analysed as a "housing-week" variable (week1 to 6 in a cubicle pen=C1-C6; week7 to 10 in a straw yard=S1-S4; **Figure 3. 2**), as they were partially confounded with each other. The initial model included treatment, housing-week (C1-C6, S1-S4), day from weekly mixing (0-6), parity (M, P), lameness score (lame, non-lame) as fixed effects and cow as a random effect. Interactions between treatment and each of the factors were fitted by backward stepwise selection. The final model included the variables of main interest and significant interactions in the fixed effects.

3.2.4.4. Feed-face occupancy

Feed-face occupancy (i.e. the percentage of available feeding space occupied by cows) was calculated from the 5-min scan sampling. The number of cows at the feed-face in the cubicle pens was divided by the number of yokes and was multiplied by 100. A theoretical space allowance of 0.6m was set for each cow in the post-and-rail feed-face in the straw yards, and a theoretical number of cows that the feed-face could accommodate was calculated for each week (e.g. when feed space was 3.6m length, theoretically six cows can feed at the same time). The number of cows observed at the feed-face was divided by the theoretical number of cows that the feed-face could accommodate, and multiplied by 100.

The percentage of cows at the feed-face at each of the 5-min scan points was calculated by dividing the number of cows observed at the feed-face by the number of cows in the group multiplied by 100. Space allowance per cow at the feed-face (i.e. the number of yokes per cow in the cubicle pens and feed-face length per cow in the straw yards) were also calculated at each of the scan sampling points.

The data for each sampling points were then averaged across the three time periods: 0-1h (H0-1), 1-2h (H1-2) and 2-3h (H2-3) after feed delivery. Effects of treatment (H and L) on feed-face occupancy, the percentage of cows at the feed-face and the space allowance per cow in the cubicles and in the straw yards were investigated for three different time periods using REML. The models included treatment, observation day (A, B, C) and housing type (treated as factors) and group size (treated as a covariate) as fixed effects. The identity of the cows within the groups changed each observation day because of weekly regrouping (dry-off and movement of cows from the far-off to close-up group) and removal of cows after parturition. However, group composition was not of main interest, hence "experimental week × treatment" was fitted as a random effect. Interactions between treatment and each of the factors were fitted by backward stepwise selection. The final model included the variables of main interest and significant interactions in the fixed effects.

3.2.4.5. Feeding behaviour

For all the feeding behaviours, the effects of treatment, housing-week, observation day, parity, lameness score, housing type and group size on all of the feeding behaviours were analysed using REML. The model included all the variables of interest as fixed effects, and cow as a random effect. Group size was treated as a covariate, and the other variables were treated as factors. Interactions between treatment and each of the factors were fitted by backward stepwise selection. The final model included the variables of main interest and significant interactions in the fixed effects.

Latency to approach the feeding area and to start feeding

Latency to first appear at the feeding area and latency to start feeding showed a heavily skewed distribution because the data contained a large number of zero observations, and even with a logit transformation, the data did not fulfil the assumption of normality. Therefore, latencies were converted to binary data to indicate whether the cow appeared at the feeding area within five minutes after fresh feed delivery (≤5 min after feed delivery, Yes=1, No=0), and whether the cow started feeding within five minutes after fresh feed delivery (≤5min after feed delivery, Yes=1, No=0). The data were analysed with GLMM using a binomial distribution with a logit link function.

The time taken for a cow to complete 60 minutes of total meal duration

The data from 5-min scan sampling were used to determine if a cow was recorded feeding 12 times at feeding (equivalent to approximately 60 minutes of meal duration) within 1h, 2h and 3h after feed delivery (60-min meal time within H0-1, H0-2, H0-3 after feed delivery, Yes=1, No=0). The cut-off threshold for meal duration was set at 60 minutes based on a study by Huzzey et al. (2006) which reported that average daily feeding time for transition cows was approximately 240 minutes and 22-28% (equivalent to 53-67 minutes) was spent during the three hours after feed delivery. GLMM was used for this analysis using a binomial distribution with a logit link function.

Time spent feeding/standing in the feed alley during the 3h after feed delivery

Effects of treatment and other variables of interest on the time spent feeding and time spent standing in the feed alley during the 3h after feed delivery were investigated. Since the precise time a cow spent at the feeding area was not measured, the data from the 5-min scan sampling were used to estimate this time (minutes). To this end, the number of times a cow was recorded as feeding and as standing in the feed alley in the observation period were multiplied by 5. Time spent feeding in the H0-1, H0-2, H0-3 after feed delivery were analysed using REML. Time spent standing in the feed alley during H0-3 after feed delivery was also analysed with REML after a logit transformation. The number of times a cow was recorded as feeding in the H0-1, the H1-2 and the H2-3 periods was also analysed to assess any change in feeding time during the first 3h after feed delivery. Transformations of the data for the H1-2 and the H2-3 periods did not fulfil the assumption of normality, and so GLMM was used for this analysis with a binomial distribution (binomial total=12) using a logit link function.

3.2.4.6. Social interactions at feed-face

Group comparison

The total number of agonistic interactions per cow was used to assess competitive behaviour at the feed-face in the treatment groups. The number of actions (push/butt + block + threat) and the number of displacements were analysed using REML following a logit transformation of the data. Non-aggressive interactions were infrequently observed, and even transformation of the data did not fulfil the assumption of normality. Therefore, the data were converted to binomial data (non-aggressive interactions were observed=1, not observed=0), and the occurrence of non-aggressive interactions was analysed using GLMM with a logit link function. The occurrence of "voluntary leave" was dropped from the analysis due to very few observations. Models for both REML and GLMM analyses included treatment, observation day and housing type (factors) and group size (covariate) as fixed effects, and "experimental week × treatment" as a random effect. Interactions between treatment and each of the factors were fitted by backward stepwise selection. The final model included the variables of main interest and significant interactions in the fixed effects.

Individual cow competitive behaviour

To assess the competitive experience of individual cows during the experimental period, the number of social interactions a cow was involved in during the observation period was analysed. The type of social interactions analysed included the number of interactions as an actor, the number of interactions as a recipient, and the total number of social interactions a cow was involved in (both as an actor and as a recipient). The number of times an actor cow half-displaced/displaced other cows (displacements as an actor), the number of times a recipient cow was half-displaced/displaced by actor cows (displacements as a recipient) and the number of active responses a recipient cow gave and an actor cow received were also analysed. REML was used to analyse all the parameters following a logit transformation. Models included treatment, housing-week, observation day, parity, lameness score (factor) and group-size (covariate) as fixed effects, and cow as a random effect. Interactions between treatment and each of the factors were fitted by backward stepwise selection. The final model included the variables of main interest and significant interactions in the fixed effects.

3.3. Results

Test statistics, P-values, means or predicted means and standard errors of means (SEM) are reported. Degrees of freedom (*df*) for most of the analyses were equal to 1, and were specifically reported only where they were different from 1. For transformed data, means obtained were back-transformed and corresponding 95% confidence intervals [95% CIs] were reported. Medians and interquartile ranges (IQR) were reported when non-parametric analyses were conducted.

3.3.1. Descriptive data

Descriptive data on the animals used in the experiment are summarised in **Table 3.2**. At the start of the experiment, "305d MY", "MY dry-off" and "SCC dry-off" were not significantly different between treatments (305 DIM: t<0.1, df=46, P=0.999; MY dry-off: U=266, df=46, P=0.841; SCC dry-off: t=0.2, df=45, P=0.868). Treatment and parity had no significant effect on the length of the dry period (treatment: $F_{1,45.0}$=0.5, P=0.505, parity: $F_{1,45.0}$<0.1, P=0.969) or the gestation period (treatment: $F_{1,45.0}$<0.1, P=0.914; parity: $F_{1,45.0}$<0.1, P=0.787). No significant treatment difference was found at any of the time points in BCS ($F_{2,92.0}$=1.0, P=0.375). There was also no treatment difference in the proportion of lame cows at any time point ($F_{2,92.0}$=1.7, P=0.185). "MY first month" and "SCC first month" ($\times 10^3$) were not different between the treatments (MY first month: $F_{1,42.0}$=0.6, P=0.441, SCC first month: $F_{1,41.0}$ =1.4, P=0.242), and were not affected by parity (MY first month: $F_{1,42.0}$=2.5, P=0.119; SCC first month: $F_{1,41.0}$=0.6, P=0.439). "SCC dry-off" did not have a significant effect on "SCC first month" ($F_{1,41.0}$=0.1, P=0.760).

No effect of treatment or parity was found in the corrected colostrum Ig level (treatment: $F_{1,32.0}$=0.1, P=0.806; parity: P: 1058±3, M: 1059±2 mg/ml, $F_{1,32.0}$=0.1, P=0.798).

Table 3.2. Descriptive data from dry-off, during the experimental period and during the first 30 days postpartum from cows in this experiment. The numbers in the table indicate means±SEM, medians (IQR) or back-transformed means [95% CIs].

	High stocking	Low stocking	P-value
Dry-off			
305d Milk yield (kg)	9976±345	9977±484	0.999
Milk yield at dry-off (kg)	23.2 (12.7-25.5)	21.5 (16.4-26.6)	0.841
Somatic cell count ($\times 10^3$)	106.7 [76.3, 149.1]	102.6 [75.4, 139.5]	0.868
Dry period			
Body condition score			
dry-off	2.45±0.09	2.41±0.09	0.375
transition	2.43±0.09	2.40±0.09	
pre-calving	2.43±0.09	2.42±0.09	
Lameness score†			
dry-off	0.17 [0.05, 0.46]	0.46 [0.18, 0.76]	0.185
transition	0.22 [0.07, 0.53]	0.13 [0.03, 0.43]	
pre-calving	0.17 [0.05, 0.46]	0.13 [0.03, 0.43]	
Lactation period			
Corrected colostrum Ig (mg/ml)	1058±2	1059±2	0.806
Milk yield first 30d (kg)	38.5±1.6	36.7±1.6	0.441
Somatic cell count first- count record ($\times 10^3$)	81.5 [55.0, 120.6]	58.2 [38.8, 87.4]	0.242
Gestation length	279.4±1.2	279.6±1.3	0.914
Dry period length	59.7±1.3	60.8±1.3	0.505

†The mean probability of cows with LS≥3 and [95% CIs] are presented.

Three cows were treated with tylosin (Tylan® 200, Elanco Animal Health, IN, USA) during the dry period due to severe lameness (H: n=1, L: n=2). Stocking density during the dry period did not significantly affect the number of cows that received veterinary treatments during the first 30 days of the subsequent lactation period (H: n=10, L: n=7, Chi-square value=0.48, P=0.498). The numbers of cows that developed mastitis, lameness, peripartum diseases and metabolic diseases are summarised in **Table 3.3**. Four cows received treatments for more than one disease (H: n=2, L: n=2).

Table 3.3. Disease incidence during the first 30 days postpartum. The numbers in the table indicate the number of animals treated, and the asterisks indicate that the numbers include animals treated for more than one disease (*).

	High stocking	**Low stocking**
Milk fever	4*	4*
Retained Placenta	1*	1*
Puerperal fever	2*	0
Mastitis	3*	1
Lameness	1	3*
Ketosis	0	1*
Displaced Abomasum	1	1*
Downer cow	2*	1*
Culled	0	1*

3.3.2. Physiological parameters

3.3.2.1. Faecal glucocorticoid metabolites

The concentrations of FGCM at different points of the dry period are shown in **Figure 3.3**. No significant effects of treatment and parity were found in the concentration of FGCM (H: 289.7 ng/g [249.6, 336.3], L: 335.0 ng/g [287.3, 390.5], $F_{1, 45.2}$=1.8, P=0.183; P: 285.6 ng/g [240.7, 339.2], M: 339.6. ng/g [298.0, 387.1], $F_{1,45.6}$=2.3, P=0.130), but there was a significant effect of sampling day ($F_{4,176.7}$=85.6, P<0.001). The concentrations of FGCM were elevated during the dry period compared to those at dry-off (dry-off: 184.1 ng/g [80.1, 423.1]; week2: 379.3 ng/g [161.1, 893.1]; week5: 323.6 ng/g [140.8, 743.7]; transition: 368.1 ng/g [160.1, 846.1]; pre-calving: 357.3 ng/g [150.0, 851.2]). There was no significant interaction between treatment and sampling time ($F_{4,172.9}$=3.9, P=0.422).

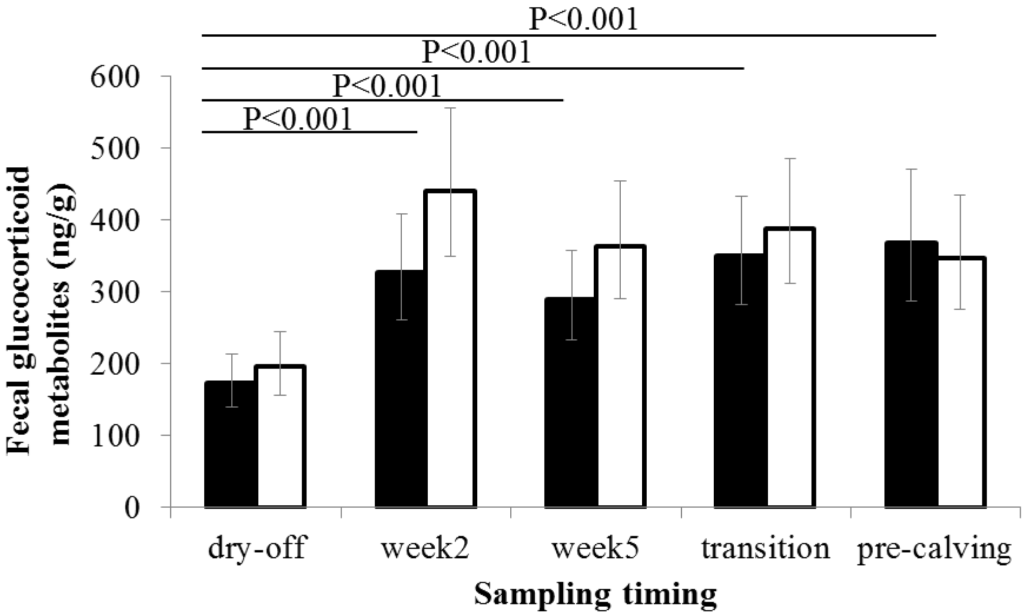

Figure 3.3. Mean concentrations of faecal glucocorticoid metabolites at the five sampling points. Black and white bars indicate High and Low stocking groups, respectively. Error bars indicate 95% CIs.

3.3.2.2. Plasma glucose, serum NEFA and BHB

The metabolic status of the cows (Glu1, Glu2, NEFA1, NEFA2, BHB1, BHB2) was not affected by stocking density (**Table 3.4**). An effect of parity was found in the concentration of plasma glucose, where plasma Glu1 was significantly higher in multiparous cows than in primiparous cows ($F_{1,37.0}$=7.5, P=0.010). However, no effect of parity was observed on the concentrations of plasma Glu2, serum BHB1, BHB2, NEFA1 or NEFA2. In both treatment groups, plasma glucose and serum BHB levels decreased from dry-off to pre-calving, whereas serum NEFA levels increased from dry-off to pre-calving. Glu2-1 and NEFA2-1 were not affected by treatment or parity.

When the change in LS was included in the model, BHB2-1 in multiparous cows was significantly greater than in primiparous cows (M: -0.24±0.06 mmol/L, P: -0.03±0.07 mmol/L, $F_{1,26.0}$=5.4, P=0.028). However, predicted means calculated from this model may not be accurate due to an unbalanced data distribution (i.e. no multiparous cows increased their LS from dry-off to pre-calving). When a model was fitted without the change in LS, no significant effect of parity on BHB2-1 was shown (**Table 3.4**). The percentage of cows that had NEFA2 concentrations higher than 0.50 mEq/L and 0.30 mEq/L was not significantly different between treatments (>0.50 mEq/L: H: 44.4%, L: 44.4%, Chi-square value<0.1, P=1.00; >0.30 mEq/L: H: 77.8%, L: 88.9%, P=0.658 *Fisher's exact test*). There was also no significant difference between treatments in the percentage of cows that had BHB2 concentrations higher than 0.60 mmol/L (H: 22.2%, L: 11.1%, P=0.658 *Fisher's exact test*).

Table 3.4. Metabolic profile at dry-off (Glucose1, NEFA1, BHB1), pre-calving (Glucose2, NEFA2, BHB2) and change from pre-calving to dry-off (Glucose2-1, NEFA2-1, BHB2-1). Data presented as means±SEM for Glucose, NEFA 2-1, BHB1 and BHB2-1, or back-transformed means [95% CIs] for NEFA1, NEFA2, and BHB2.

	High stocking	**Low stocking**	**Primi parous**	**Multi parous**	**P value**	
					Treatment	**Parity**
Glucose 1	3.43±0.06	3.45±0.07	3.31±0.08	3.58±0.06	0.857	**0.010**
Glucose 2	3.13±0.06	3.11±0.05	3.05±0.06	3.19±0.05	0.812	0.087
Glucose 2-1 (mmol/L)	-0.34±0.08	-0.23±0.09	-0.29±0.11	-0.28±0.07	0.384	0.892
NEFA 1	0.16 [0.12, 0.21]	0.14 [0.10, 0.18]	0.13 [0.09, 0.18]	0.17 [0.14, 0.22]	0.414	0.146
NEFA 2	0.43 [0.34, 0.54]	0.46 [0.37, 0.57]	0.45 [0.34, 0.59]	0.44 [0.36, 0.52]	0.682	0.846
NEFA 2-1 (mEq/L)	0.27±0.07	0.38±0.08	0.39±0.10	0.26±0.06	0.278	0.260
BHB 1	0.61±0.04	0.63±0.05	0.57±0.04	0.66±0.05	0.676	0.157
BHB 2	0.47 [0.11, 1.95]	0.44 [0.11, 1.69]	0.47 [0.40, 0.55]	0.44 [0.39, 0.49]	0.403	0.479
BHB2-1 (mmol/L)	-0.09±0.06	-0.17±0.07	-0.11±0.08	-0.15±0.05	0.307	0.655

3.3.3. Activity levels of cows

The final models used to analyse the activity levels of cows were summarised in **Table 3.5**.

Table 3.5. Final models used for the analyses of the activity levels of cows.

Final models for activity levels of cows	
MotionIndex†	*treatment + treatment.housing-week + housing-week + days from mixing + parity + lameness score*
Step count†	*treatment + treatment.housing-week + treatment.lameness score + housing-week + lameness score + days from mixing + parity*
Lying bout†	*treatment + housing-week + days from mixing + parity + lameness score*
Lying proportion	*treatment + housing-week + days from mixing + parity + lameness score*

†The data were analysed after a logit transformation

Treatment

There was no significant difference between H and L groups in MI (H:2489/day [2263, 2736], L: 2438/day [2207, 2692], $F_{1,44.6}$<0.1, P=0.893), SC (H: 893/day [816, 978], L: 952/day [867, 1047], $F_{1,43.6}$=0.6, P=0.454), LB (H: 9.0/day [8.2, 9.7], L: 8.8/day [8.0, 9.6], $F_{1,44.8}$=0.1, P=0.670), and LP (H: 0.58±0.01, L: 0.59±0.02, $F_{1,44.2}$<0.1, P=0.911). However, a significant interaction between treatment and housing-week was noted in MI ($F_{9,2433.5}$=2.4, P=0.012) and SC ($F_{9,2383.1}$=3.7, P<0.001). There was also a significant interaction between treatment and lameness score in SC ($F_{1,1126.1}$=5.3, P=0.022). Further details of these results are reported below.

Parity

A significant effect of parity was found for MI ($F_{1, 45.2}$=16.0, P<0.001) and SC ($F_{1, 43.8}$=9.2, P=0.004) with primiparous cows having higher MI (2818/day [2529, 3141]) and SC (1042/day) [944, 1151] than multiparous cows (MI: 2143 [1967, 2335], SC: 855/day [788, 927]). No significant effect of parity was found in LB (M: 8.1/day [7.6, 8.8], P: 9.0/day [8.2, 9.9], $F_{1, 45.3}$=1.7, P=0.202) or LP (M: 0.57±0.01, P: 0.56±0.02, $F_{1, 44.7}$=0.1, P=0.737).

Housing types and changes over week

Figure 3.4 shows the weekly changes in the activity levels of cows in each of the housing types. A significant interaction between treatment and housing-week was found in MI ($F_{9,2433.5}$=2.4, P=0.012) and SC ($F_{9,2383.1}$=3.7, P<0.001). In the H group, MI and SC significantly increased from C1 to C2 (MI: *t*=3.4, P=0.002, SC: *t*=3.2, P=0.003) followed by a significant decrease in C4 (MI: *t*=2.7, P=0.009, SC: *t*=2.2, P=0.031). MI and SC in the L group showed a significant increase from C1 to C3 (MI: t=2.1, P=0.042, SC: t=3.0, P=0.005) and stabilised at around 2500/day (MI) and 1000 steps/day (SC) until C6, leading to a significant treatment difference in step count in C4 (t=2.7, P=0.011). MI in both groups increased again from C6 to S1, although it returned to the same level as C6 in S2. SC in the L group significantly decreased from C6 to S1 (t=3.5, P=0.001) and remained lower until S4, whilst SC in the H group showed a gradual decrease from C5 to S3. Irrespective of the treatment groups, a significant effect of housing-week was found in LB ($F_{9,2440.7}$=29.8, P<0.001) and LP ($F_{9,2439.3}$=87.1, P<0.001), with a significant increase from C1 to C2 (LB: t=3.9, P<0.001; LP: t=4.9, P<0.001) and from C6 to S1 (LB: t=10.5., P<0.001; LP: t=14.7, P<0.001).

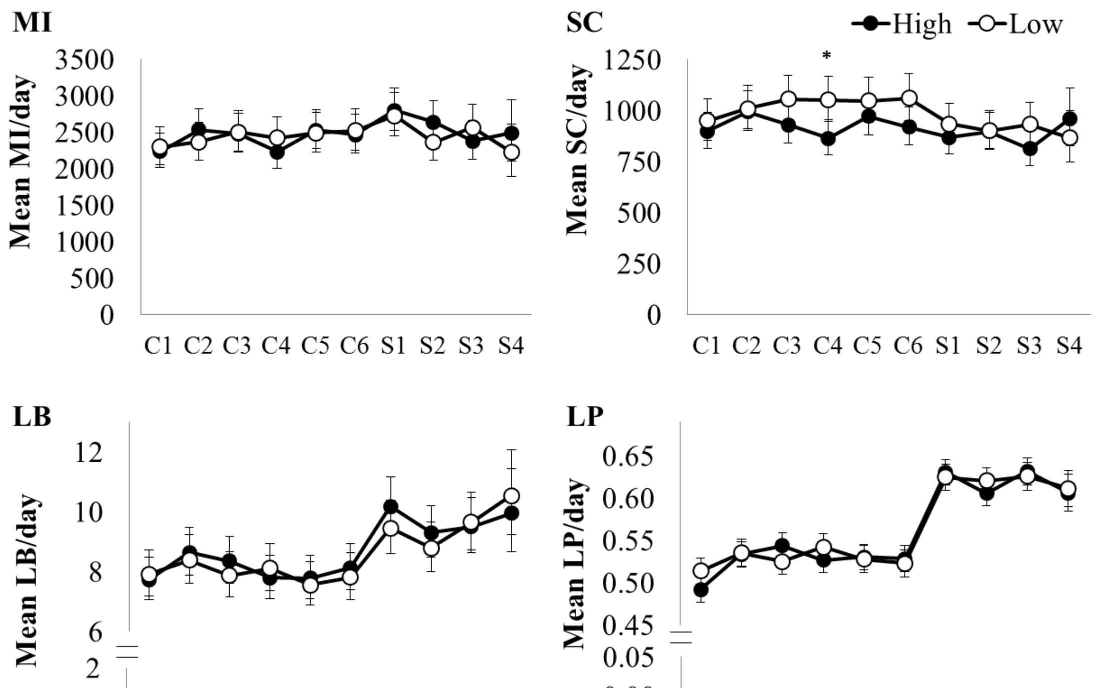

Figure 3.4. Changes over week in daily MotionIndex (MI), step count (SC), lying bouts (LB) and lying proportion (LP) in the cubicle pen (C1-C6) and the straw yard (S1-S4). Error bars indicate 95% CIs for MI, SC and LB, and SEM for LP. Asterisk signifies treatment differences at P<0.05.

Changes in activity levels of cows over the days following weekly regrouping

A significant effect of days from mixing was found in MI ($F_{6,\ 2425.0}$=34.1, P<0.001), SC ($F_{6,\ 2373.1}$=8.4, P<0.001), LB ($F_{6,\ 2434.2}$=4.12, P<0.001) and LP ($F_{6,\ 2433.6}$=6.0, P<0.001) (**Figure 3.5**). MI showed a significant decrease on the day after mixing (*t*=8.1, P<0.001), followed by a gradual decline until the third day after mixing. SC followed a similar pattern to MI, with a significant decrease on the day after mixing (*t*=2.6, P=0.009), and a further decline until the third day after mixing. However, both MI and SC increased on the fifth and sixth day after mixing. LB significantly increased after mixing (*t*=4.0, P<0.001) for two days but declined to the initial level at around 8.75/day until the sixth day after mixing. LP also significantly increased after mixing (*t*=4.0, P<0.001), and remained higher than on the day of mixing for the following six days.

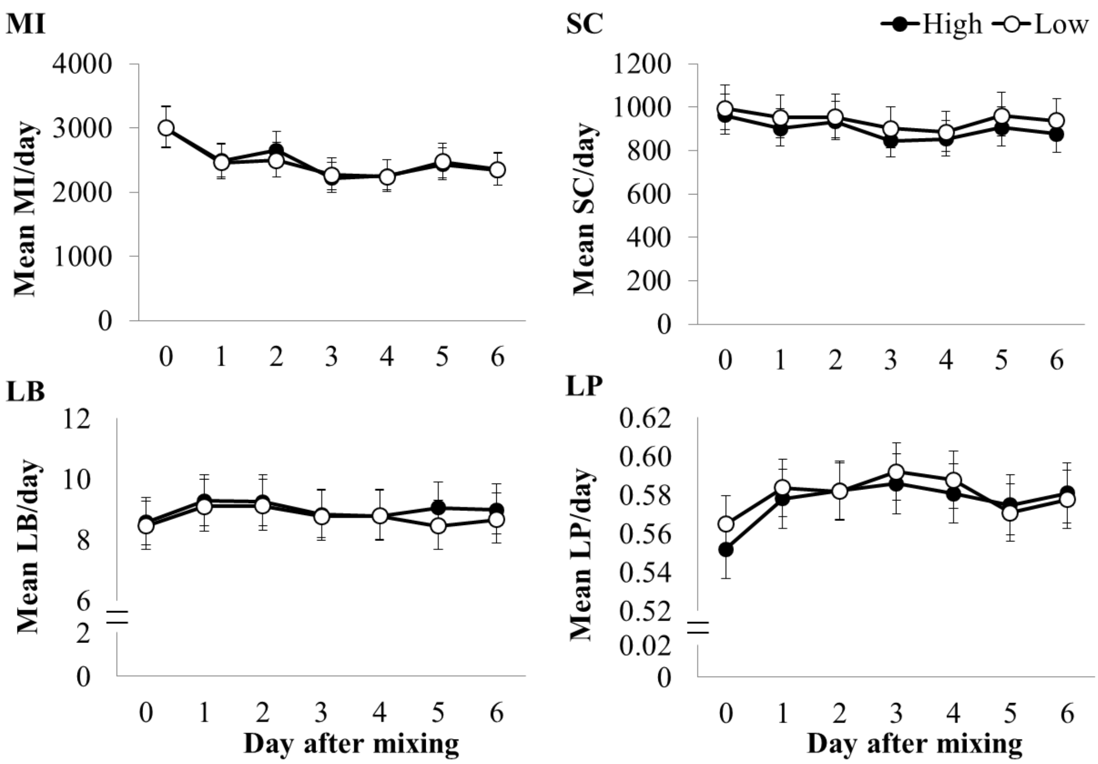

Figure 3.5. Daily changes in MotionIndex (MI), step count (SC), lying bouts (LB) and lying proportion (LP) from weekly dry-off. Error bars indicate 95% CIs for MI, SC and LB, and SEM for LP.

Lameness score

Significantly lower MI was observed in lame cows compared to non-lame cows (lame: 2350 [2166, 2548], non-lame: 2570 [2402, 2750]/day, $F_{1,\ 1356.6}$=7.2, P=0.007). No significant difference in SC was found between the lame (989/day [879, 1112]) and the non-lame cows (968/day [881, 1064]) in the L group, whilst significantly lower SC was observed in the lame cows (859/day [778, 949]) compared to the non-lame cows (964/day [81, 1055]) in the H group (t=3.4, P=0.001). There was no significant effect of lameness score on LB (non-lame 8.4/day [7.9, 9.0], lame: 8.8/day [8.2, 9.5], $F_{1,\ 1648}$=3.5, P=0.202) or LP (non-lame: 0.56±0.01, lame: 0.57±0.01, $F_{1,\ 1809.0}$<0.1, P=0.630).

3.3.4. Feed-face occupancy

The final models used to analyse the feed-face occupancy, the percentages of cows at the feed-face and the space allowance per cow are summarised in **Table 3.6**.

Table 3.6. The final models used to analyse the occupancy of the feeding space in the H0-1, H1-2 and H2-3 periods after feed delivery.

Final models for occupancy of the feeding space	
Feed-face occupancy	
H0-1	*treatment + treatment.housing + housing + observation + group size*
H1-2	*treatment + observation + housing + group size*
H2-3	*treatment + observation + housing + group size*
Percentage of cows at feed-face	
H0-1	*treatment + treatment.housing + housing + observation + group size*
H1-2	*treatment + observation + housing + group size*
H2-3	*treatment + observation + housing + group size*
Space allowance per cow (yoke, post-and-rail)†	
H0-1	*treatment + observation + group size*
H1-2	*treatment + observation + group size*
H2-3	*treatment + observation + group size*

†Space allowance per cow for both the yoke and post-and-rail feed-face were analysed following a logit transformation.

3.3.4.1. Feed-face occupancy

Feed-face occupancy (the percentage of available feeding space occupied by cows) in the 3h after feed delivery for the two types of feed-faces in each of the treatment groups are shown in **Figure 3.6**. A significant treatment effect was found on feed-face occupancy ($F_{1,80.0}$=33.5, P<0.001), with a significant interaction between treatment and housing type during the H0-1 period ($F_{1,263.0}$ =10.9, P=0.001). The feed-face in the H group was significantly more crowded than in the L group, regardless of the feed barrier designs used (P<0.001). However, the feed-face in the straw yard (post-and-rail feed barrier) in the H group was more crowded than the feed-face in the cubicle pen (yoke feed barrier, t=3.9, P<0.001), whilst this difference was not seen in the L group.

During the H1-2 and the H2-3 periods, the feed-face in H group was more crowded than in the L group (H1-2: $F_{1,79.9}$=102.2, P<0.001; H2-3: $F_{1,81.1}$=197.9, P<0.001), and the yoke feed-face was more crowded than the post-and-rail feed-face during the H2-3 period ($F_{1,250.7}$=7.9, P=0.005). A significant effect of observation day was found during the H0-1 ($F_{2,223.2}$=9.8, P<0.001), the H1-2 ($F_{2,221.2}$=13.6, P<0.001), and the H2-3 ($F_{2,212.6}$=3.9, P=0.023) periods. Observation day C was significantly more crowded than observation day A and B during the H0-1 (A: t=4.3, P<0.001; B: t=2.9, P=0.004) and the H1-2 periods (A: t=4.7, P<0.001; B: t=4.3, P<0.001), and observation day B had a lower density than observation day A (t=2.0, P=0.044) and C (t=2.6, P=0.009) during the H2-3 period.

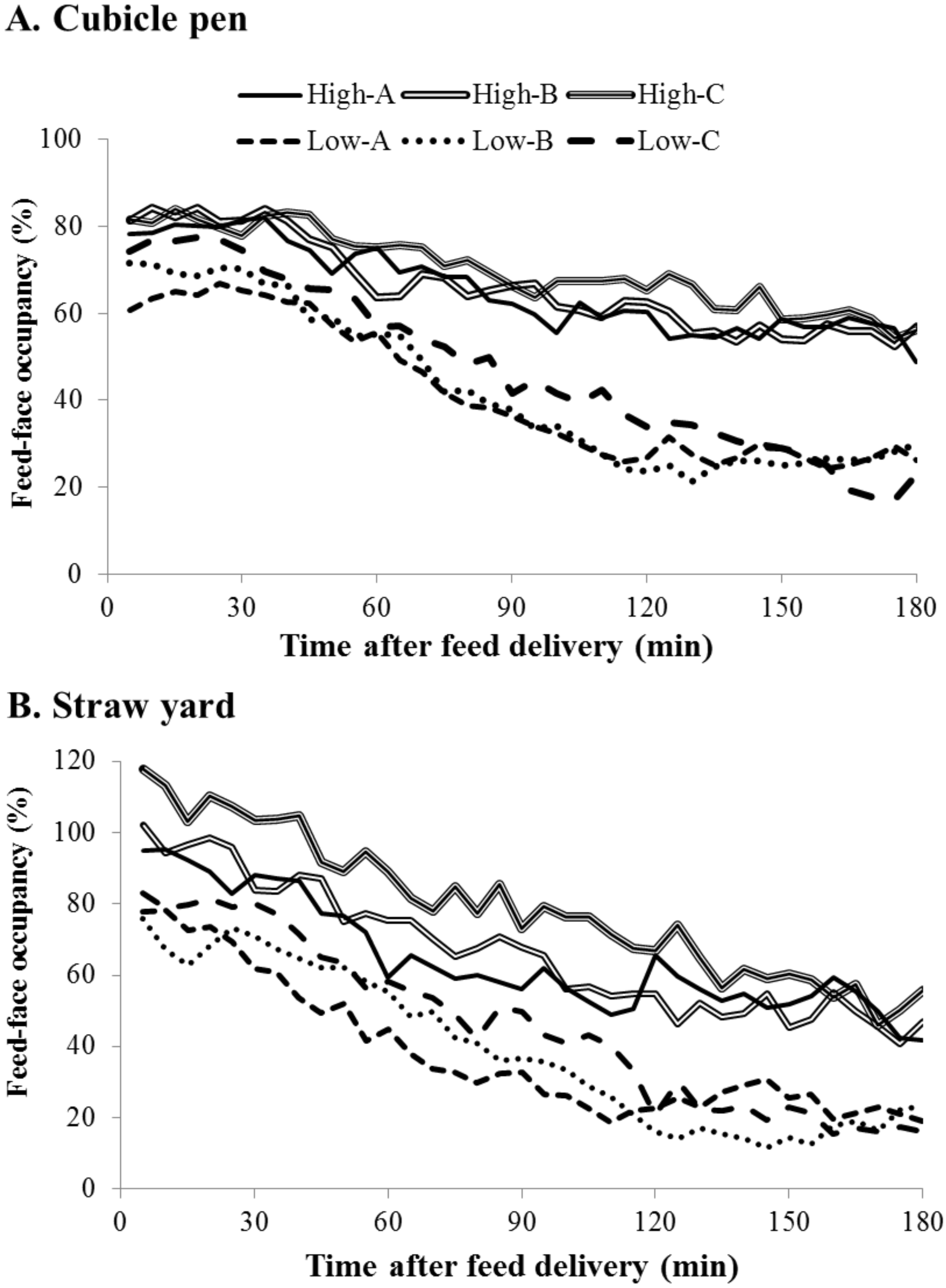

Figure 3.6. Feed-face occupancy (%) in the three hours after feed delivery in the cubicle pen (A) and the straw yard (B). Different types of solid lines represent the High stocking group on observation A, B and C. Different types of dotted lines represent the Low stocking group on observation day A, B and C.

3.3.4.2. Percentage of cows at feed-face

Figure 3.7 shows the percentage of cows observed at the feed-face during the 3h after feed delivery for both types of feed-face in each of the treatment groups. During the H0-1 period, a significantly higher percentage of cows was observed at the feed-face in the L group than in the H group ($F_{1,81.0}$=90.1, P<0.001). However a significant interaction was also found between treatment and housing type ($F_{1,263.9}$=4.1, P=0.045), where a lower percentage of cows was observed at the yoke feed-face than the post-and-rail feed-face in the H group (t=2.7, P=0.006). No difference in the housing type was observed in the L group. There was also a significant effect of observation day on the percentage of cows at the feed-face during the H0-1 period ($F_{2,223.8}$=7.8, P<0.001), where a higher percentage of cows was at the feed-face during observation C compared to observation A (t=3.9, P<0.001) and B (t=2.5, P=0.013).

During the H1-2 period, a significantly higher percentage of cows were at the feed-face in the L group compared to in the H group ($F_{1,82.2}$=10.6, P=0.002), and a significant effect of observation day was found ($F_{2,223.5}$=11.3, P<0.001). A higher percentage of cows were at the feed-face on observation day C than observation day A (t=4.3, P<0.001) and B (t=4.0, P<0.001). No significant effect of housing type was found in the percentage of cows at the feed-face during the H1-2 period ($F_{1,267.3}$<0.1, P=0.949). In contrast, a significantly higher percentage of cows were observed at the feed-face in the H group compared to the L group during the H2-3 period ($F_{1,80.9}$=8.4, P=0.005), and significantly more cows were at the feed-face on observation day A and C than observation day B ($F_{2,212.6}$=3.3, P=0.039). A significant effect of housing type was found during the H2-3 period ($F_{1,250.9}$=11.2, P=0.001), where a higher percentage of cows were observed at the yoke feed-face (29.0±1.1%) compared to the post-and-rail feed-face (23.1±1.2%). No significant effect of group size was found in the percentage of cows at the feed-face in the 3h after feed delivery (H0-1: $F_{1,259.6}$=2.0, P=0.164; H1-2: $F_{1,245.7}$=0.3, P=0.583; H2-3: $F_{1,249.3}$=3.1, P=0.080).

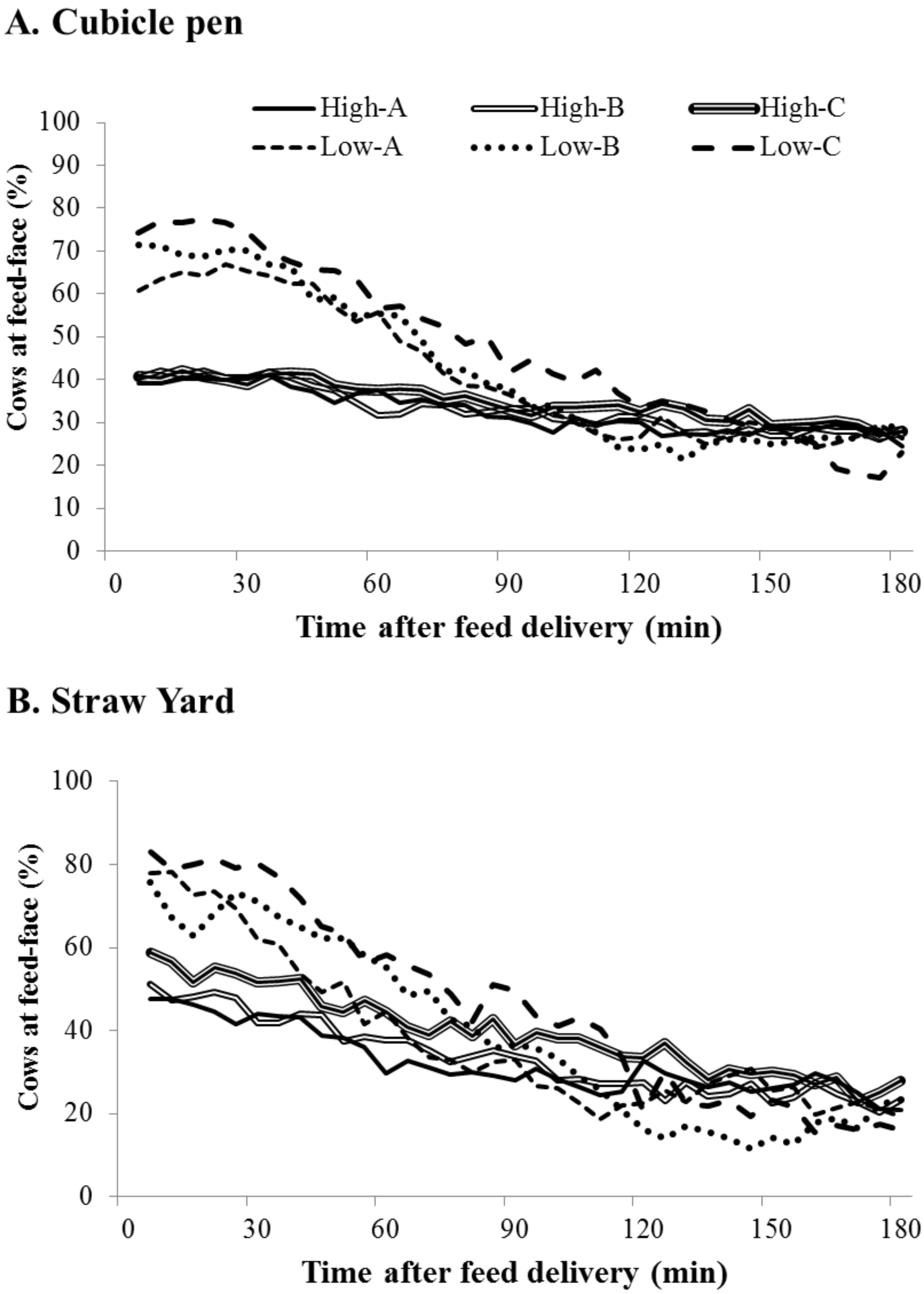

Figure 3.7. Percentages of cows at the feed-face in the three hours after feed delivery in the cubicle pen (A), and the straw yard (B). Different types of solid lines represent the High stocking group on observation A, B and C. Different types of dotted lines represent the Low stocking group on observation day A, B and C.

3.3.4.3. Space availability at the feed-face

The number of yokes available per cow and the feed-face length available per cow in the 3h after feed delivery are shown in **Figure 3.8.** Significantly more yokes and wider feeding space were available per cow in the L group compared to the H group in the H0-1 (yoke: $F_{1,48.4}$=6.8, P=0.012, post-and-rail: $F_{1,45.2}$=13.0, P<0.001), the H1-2 (yoke: H1-2: $F_{1,46.6}$=56.6, P<0.001, post-and-rail: H1-2: $F_{1,43.2}$=41.0, P<0.001) and the H2-3 periods (yoke: $F_{1,47.4}$=114.7, P<0.001, post-and-rail: $F_{1,43.2}$=164.8, P<0.001). In the cubicle pen, a significant effect of observation day was found in the H1-2 period ($F_{2,96.6}$=8.2, P=0.020), where more yokes were available per cow on observation day A (t=2.2, P=0.029) and B (t=2.7, P=0.008) compared to observation day C.

In the straw yard, a significant effect of observation day was found in all of the periods (H0-1: $F_{2,89.6}$=5.8, P=0.004; H1-2: $F_{2,85.8}$=8.8, P<0.001; H2-3: $F_{2,80.1}$=3.9, P=0.025). Fewer feeding space was available per cow on observation day C than observation day A (H0-1: t=6.0, P<0.001; H1-2: t=3.9, P<0.001) and B (H0-1: t=7.4, P<0.001; H1-2: t=3.9, P=0.001) in the H0-1 and H1-2 periods. In the H2-3 period, each cow had more feeding space on observation day B than observation day A (t=2.4, P=0.042) and C (t=2.3, P=0.049). A significant effect of group size was only seen in the post-and-rail feed-face in the H2-3 period, where 0.05m [95% CIs: 0.04, 0.06] wider feeding space became available per cow as group size increased ($F_{1,52.9}$=70.0, P<0.001).

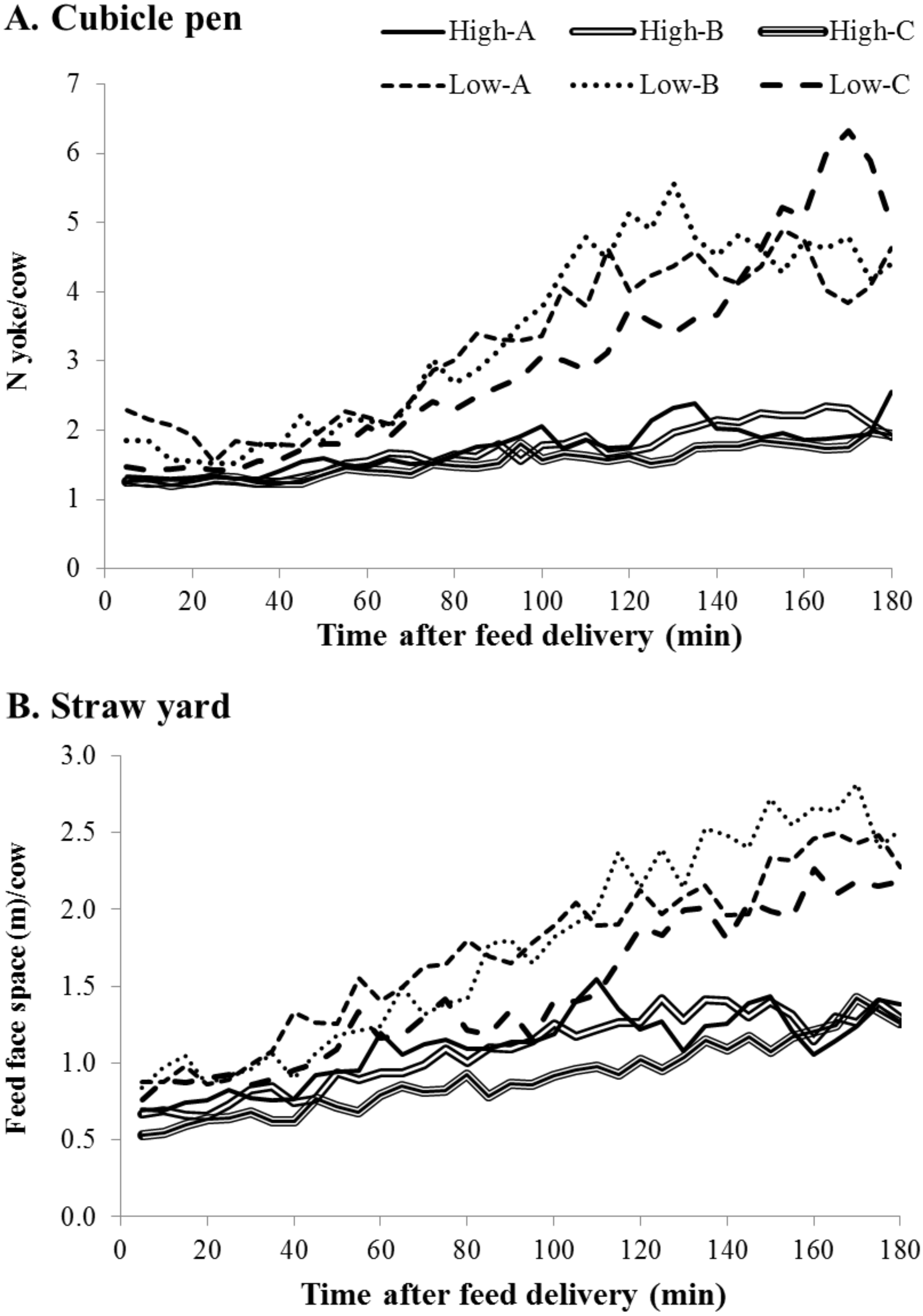

Figure 3.8. Feed-face space availability per cow in the three hours after feed delivery in the cubicle pen (A), and the straw yard (B). Different types of solid lines represent the High stocking group on observation A, B and C. Different types of dotted lines represent the Low stocking group on observation day A, B and C.

3.3.5. Feeding behaviour

The final models used to analyse feeding behaviour during the three hours after feed delivery were summarised in **Table 3.7**.

Table 3.7. Statistical tests and final models used to analyse feeding behaviour during the three hours after feed delivery.

Final models for feeding behaviour	
Cows appeared at feeding area ≤5min after feed delivery	
	treatment + housing-week + observation day + parity + lameness score + group size
Cows started feeding ≤5min after feed delivery	
	treatment + treatment.lameness score +housing-week + observation day + parity + lameness score + group size
60-min meal after feed delivery	
<H0-1	*treatment + treatment.houing-week + housing-week + observation day + parity + lameness score + group size*
<H0-2	*treatment + housing-week + observation day + parity + lameness score + group size*
<H0-3	*treatment + housing-week + observation day + parity + lameness score + group size*
Time spent on feeding in the H0-1, H0-2, H0-3 after feed delivery	
	treatment + housing-week + observation day + parity + lameness score + group size
Time spent on standing in the feed alley in the H0-3 after feed delivery †	
	treatment + treatment.housing-week + housing-week + observation day + parity + lameness score + group size
N times a cow was observed feeding in the H0-1, H1-2, H2-3 periods	
H0-1	*treatment + treatment.lameness score + housing-week + observation day + parity + lameness score + group size*
H1-2	*treatment + housing-week + observation day + parity + lameness score + group size*
H2-3	*treatment + housing-week + observation day + parity + lameness score + group size*

†The data were analysed after a logit transformation.

3.3.5.1. Probability of cows approaching the feeding area, and starting to feed within five minutes after feed delivery

Treatment and lameness score

The probability of cows approaching the feeding area ≤5 min after feed delivery was significantly higher in the L group than in the H group (L: 0.92 [0.85, 0.96], H: 0.73 [0.61, 0.82], $F_{1,58.3}$=12.7, P<0.001). There is also a significant difference between treatment groups in the probability of cows starting to feed ≤5 min after feed delivery (L: 0.92 [0.86, 0.96], H:

0.60 [0.47, 0.70], $F_{1,58.0}$=30.2, P<0.001). There was a significant interaction between treatment and lameness score ($F_{1,223.7}$=4.5, P=0.035), where non-lame cows in the L group were more likely to start feeding ≤5 min after feed delivery than lame cows in the L group (non-lame: 0.95 [0.90, 0.97], lame: 0.82 [0.67, 0.91], t=3.2, P=0.028). There was no significant difference in the lameness score in the H group (non-lame: 0.60 [0.47, 0.71], lame: 0.60 [0.38, 0.71]). Regardless of treatment group, lame cows were significantly less likely to approach the feeding area ≤5 min after feed delivery (non-lame: 0.84 [0.77, 0.88], lame: 0.70 [0.60, 0.80], $F_{1,277.9}$=5.7 P=0.018).

Housing types and changes over week

A significant effect of housing-week was found in the probability of cows approaching the feeding area ≤5 min after feed delivery ($F_{9,1176.9}$=4.9, P<0.001; **Figure 3.9A**) and the probability of cows starting to feed ≤5 min after feed delivery ($F_{9,1178.4}$=4.0, P<0.001; **Figure 3.9B**). There was no significant interaction between treatment and housing-week in both variables. The probability of cows approaching the feeding area and the probability of cows starting to feed gradually declined from C1 to C4, but both variables increased from C6 to S1, returning to the same level as C1. This resulted in a significant difference between the C2-C6 and the S1-S4 periods in both variables.

Observation day

Regardless of treatment group, a significant effect of observation day was found in the probability of cows approaching the feeding area ≤5 min after feed delivery ($F_{2,1165.9}$=4.2, P=0.015) and the probability of cows starting to feed ≤5 min after feed delivery ($F_{2,1164.7}$=3.9, P=0.021). The probabilities were lowest on observation day A (approaching the area: 0.79, [0.71, 0.86], starting to feed: 0.74, [0.65, 0.81]), and increased from observation day B (approaching the area: 0.84, [0.76, 0.89], starting to feed: 0.76, [0.68, 0.83]) to observation day C (approaching the area: 0.86, [0.79, 0.91], starting to feed: 0.82, [0.74, 0.87]).

Parity and group size

No effect of parity was also found on either of the variables (approaching the feeding area: $F_{1,44.2}$=2.7, P=0.112, starting to feed: $F_{1,42.2}$=1.2, P=0.288). Larger group size significantly increased the probability of cows starting to feed ≤5 min after feed delivery ($F_{1,1204.2}$=10.7, P=0.001), but group size did not affect the probability of cows approaching the feeding area ($F_{1,1207.1}$=2.4, P=0.118).

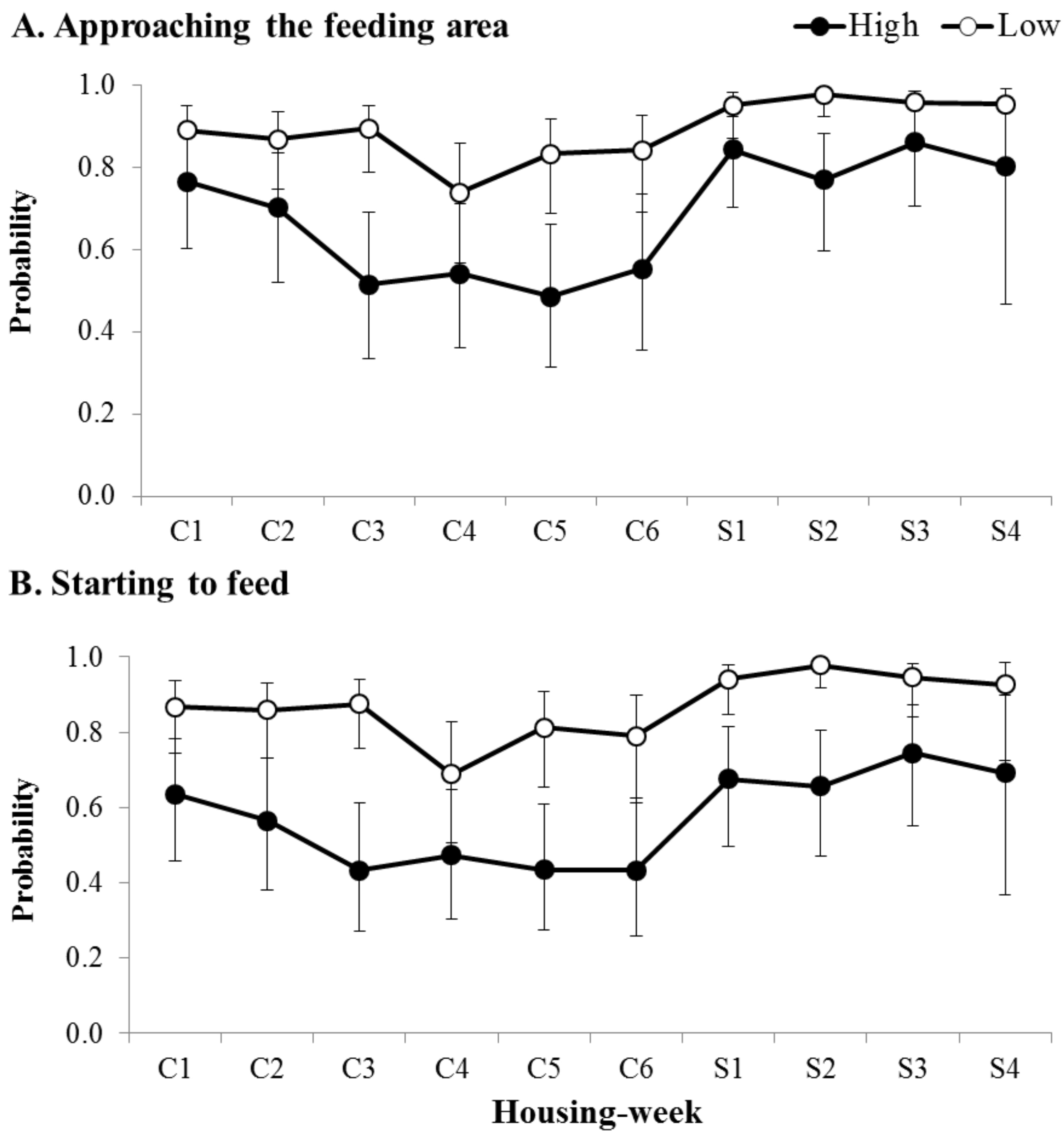

Figure 3.9. Changes over week in the mean probability of cows approaching the feeding area (A) and the probability of cows starting to feed (B) within five minutes after feed delivery in the cubicle pen (C1-C6) and the straw yard (S1-S4). Error bars indicate 95% CIs.

3.3.5.2. Probability of achieving a 60-min meal time within 1h, 2h and 3h after feed delivery

Treatment and housing-week

A significant effect of treatment was found in the probability of cows achieving a total of 60 minute meal time (60-min meal) within the H0-1 period ($F_{1,62.8}$=30.9, P<0.001). The probability was significantly higher in the L group than that in the H group (H: 0.08 [0.05, 0.14], L: 0.47 [0.32, 0.62]), but there was a significant interaction between treatment and housing-week ($F_{9,1172.3}$=2.4, P=0.012; **Figure 3.10A**). The L group had a significantly higher probability than the H group for all the weeks except C1. The probability increased from C1 to C3 in the L group, whilst there was no change in the probability in the H group. The probability of cows achieving a 60-min meal within the H0-1 period was further increased from C6 to S1 and S2 in the L group, whereas the probability in the H group was the lowest at S1. This resulted in a larger difference between the H and L groups in the probability of cows achieving a 60-min meal within the H0-1 period in the weeks when the post-and-rail feed-face was used.

The probability of cows achieving a 60-min meal within the H0-2 and the H0-3 periods was significantly lower in the H group than in the L group (H0-2: H=0.43 [0.32, 0.54], L=0.72 [0.60, 0.81], $F_{1,56.5}$=12.4, P<0.001; H0-3: H=0.66 [0.56, 0.75], L=0.80 [0.72, 0.86], $F_{1,61.8}$=5.2, P=0.027). A significant effect of housing-week was found on the probability of cows achieving a 60-min meal within the H0-2 ($F_{9,1177.8}$=3.6, P<0.001; **Figure 3.10B**) and the H0-3 ($F_{9,1181.9}$=3.0, P=0.002; **Figure 3.10C**) periods. A gradual increase in the probability was seen from C1 to C5 in the H0-2 period, followed by a further increase from S1 to S2. The probability was higher in C5 and S2 in the H0-3 period, with a significant difference to C1, C3, C6 and S1.

Observation day

There was no significant effect of observation day on the probability of cows achieving a 60-min meal within the H0-1 period ($F_{2,1155.2}$=2.6, P=0.275). However, observation day had a significant effect on the probability of cows achieving a 60-min meal within the H0-2 ($F_{2,1164.9}$=4.6, P=0.011) and the H0-3 periods ($F_{2,1166.4}$=5.1, P=0.006). The probabilities increased from observation day A (H0-2: 0.52, [0.42, 0.61], H0-3: 0.69, [0.61, 0.76]) to observation day B (H0-2: 0.57, [0.47, 0.67], H0-3: 0.73, [0.66, 0.80]), and further increased to observation day C (H0-2: 0.63, [0.53, 0.72], H0-3: 0.78, [0.71, 0.84]). Consequently, the probability on observation day C was significantly higher than that on observation day A (H0-2: t=3.0, P=0.003, H0-3: t=3.2, P=0.001).

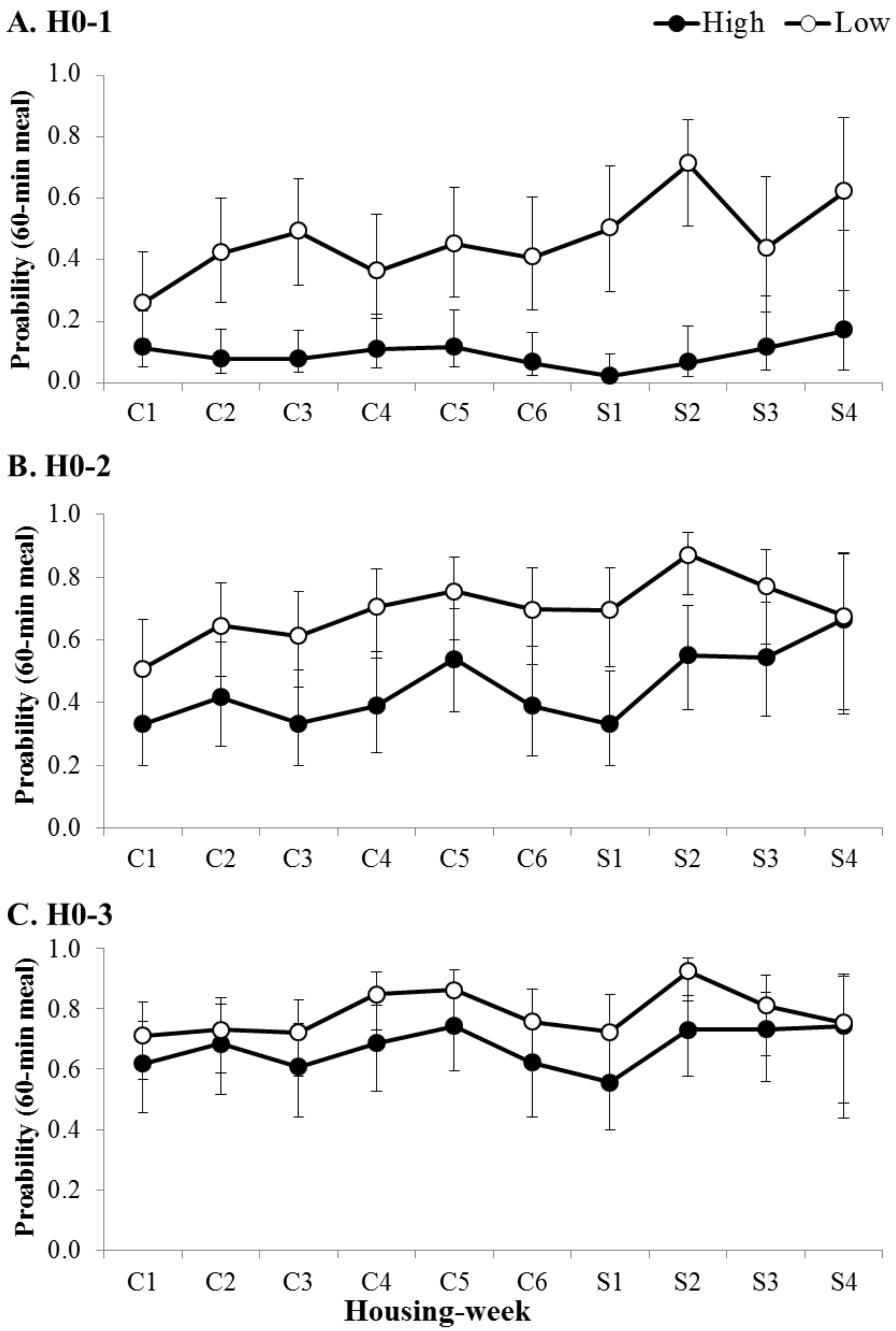

Figure 3.10. Changes over week in the mean probability of cows achieving a 60-min meal A) within the first 1 hour (H0-1), B) 2 hours (H0-2) and C) 3 hours (H0-3) after feed delivery in the cubicle pen (C1-C6) and the straw yard (S1-S4). Error bars indicate 95% CIs.

Parity

No significant effect of parity was found in the probability of cows achieving a 60-min meal within the H0-1 (P: 0.27 [0.17, 0.42], M: 0.17 [0.11, 0.26], $F_{1,41.7}$=2.1, P=0.155) or the H0-2 (P: 0.66, [0.53, 0.76], M: 0.50, [0.39, 0.60], $F_{1,43.5}$=4.0, P=0.052) periods. However, primiparous cows were more likely to achieve a 60-min meal within the H0-3 period compared to multiparous cows (P: 0.79, [0.71, 0.86], M: 0.67, [0.58, 0.75], $F_{1,45.6}$=4.5, P=0.040).

Lameness score

Lameness score did not affect the probability of cows achieving a 60-min meal within the H0-1 period (non-lame: 0.24 [0.17, 0.33], lame: 0.20 [0.12, 0.31], $F_{1,318.7}$=0.7, P=0.401). However, a significant effect of lameness score was found in the probability for the H0-2 ($F_{1,300.5}$=4.7, P=0.032) and the H0-3 ($F_{1,250.1}$=5.3, P=0.023) periods. Lame cows were less likely to attain a 60-min meal within the H0-2 (0.51, [0.39, 0.63]) and the H0-3 (0.67, [0.56, 0.77]) periods than non-lame cows (H0-2: 0.64, [0.56, 0.72], H0-3: 0.79, [0.73, 0.84]).

Group size

The probability of cows achieving a 60-min meal within the H0-1 period increased by 0.12±0.03 for each unit increase in group size ($F_{1,1152.3}$=12.7, P<0.001). The probability for the H0-2 period also increased by 0.05±0.02 for each unit increase in group size ($F_{1,1202.}$6=5.1, P=0.024), but this effect disappeared when the observation time extended to the H0-3 period after feed delivery ($F_{1,1181.5}$=1.7, P=0.198).

3.3.5.3. The number of times a cow was observed feeding

The number of times a cow was observed feeding in the H0-1, H1-2 and H2-3 periods was analysed to investigate the differences between the treatment groups in the time spent feeding. A significant effect of treatment was found on the number of times a cow was observed feeding in the H0-1 and the H2-3 periods (H0-1: $F_{1,53.3}$=32.3, P<0.001, H2-3: $F_{1,67.7}$=9.3, P=0.003). Cows in the L group were more frequently observed feeding during the H0-1 period (H: 6.1 times [4.9, 7.3], L 9.8 times [8.9, 10.5]), whilst cows in the H group were more frequently observed feeding in the H2-3 period (H: 3.7 times [3.1, 4.4], L: 2.3 times [1.8, 3.0]). No treatment difference was found in the H1-2 period (H: 4.5 [3.7, 5.4], L: 5.2 [4.3, 6.1], $F_{1,58.8}$=1.1, P=0.295).

A significant interaction between treatment and lameness score was found in the H0-1 period ($F_{1,429.5}$=4.0, P=0.045). In the L group, non-lame cows were more frequently observed feeding than lame cows (non-lame: 10.5 times [9.8, 11.0], lame: 8.9 times [7.5, 10.0], t=3.2, P=0.003), but there was no effect of the lameness score on feeding during this period for the H group (non-lame: 6.3 times [5.0, 7.5], lame: 5.9 [4.4, 7.5]).

A significant effect of housing-week was found in the H0-1 ($F_{9,1175.4}$=3.7, P<0.001), the H1-2 ($F_{9,1178.9}$=2.5, P=0.009), and the H2-3 ($F_{9,1184.7}$=3.0, P=0.002) periods. The number of times a cow was observed feeding in the H0-1 period significantly increased from C6 to S2 (t=4.5, P<0.001), whereas the number of times a cow was observed feeding in the H1-2 period showed a significant increase during the weeks in the cubicle pen (C1-C5, t=4.1, P<0.001; **Figure 3.11A**). In contrast, the number of times a cow was observed feeding in the H2-3 period gradually decreased from C4 to S2 (**Figure 3.11A**).

A significant effect of observation day was also found in the H0-1 ($F_{2,1164.3}$=4.7, P=0.009) and the H1-2 ($F_{2,1166.4}$=8.7, P<0.001) periods, but not in the H2-3 period ($F_{2,1166.0}$=1.5, P=0.221). Cows were more frequently observed as feeding on observation day C than observation day A (t=3.0, P=0.002; **Figure 3.11B**) in the H0-1 period, and on observation day C than observation day A (t=4.2, P<0.001) and B (t=2.3, P=0.023; **Figure 3.11B**) in the H1-2 period. In addition, parity had a significant effect on feeding during the H1-2 period, where primiparous cows were more frequently observed feeding than multiparous cows (P: 5.6 [4.6, 6.5], M: 4.2 [3.5, 5.0], $F_{1,44.8}$=5.2, P=0.027).

Cows were more likely to be observed feeding in the H0-1 period when the group size was larger (probability increased by 0.06±0.02 per unit increase, $F_{1,1209.7}$=15.0, P<0.001), while cows were more likely to be observed feeding in the H2-3 period when the group size was smaller (probability decreased by 0.05±0.02 per unit increase, $F_{1,1107.9}$=7.2, P=0.007).

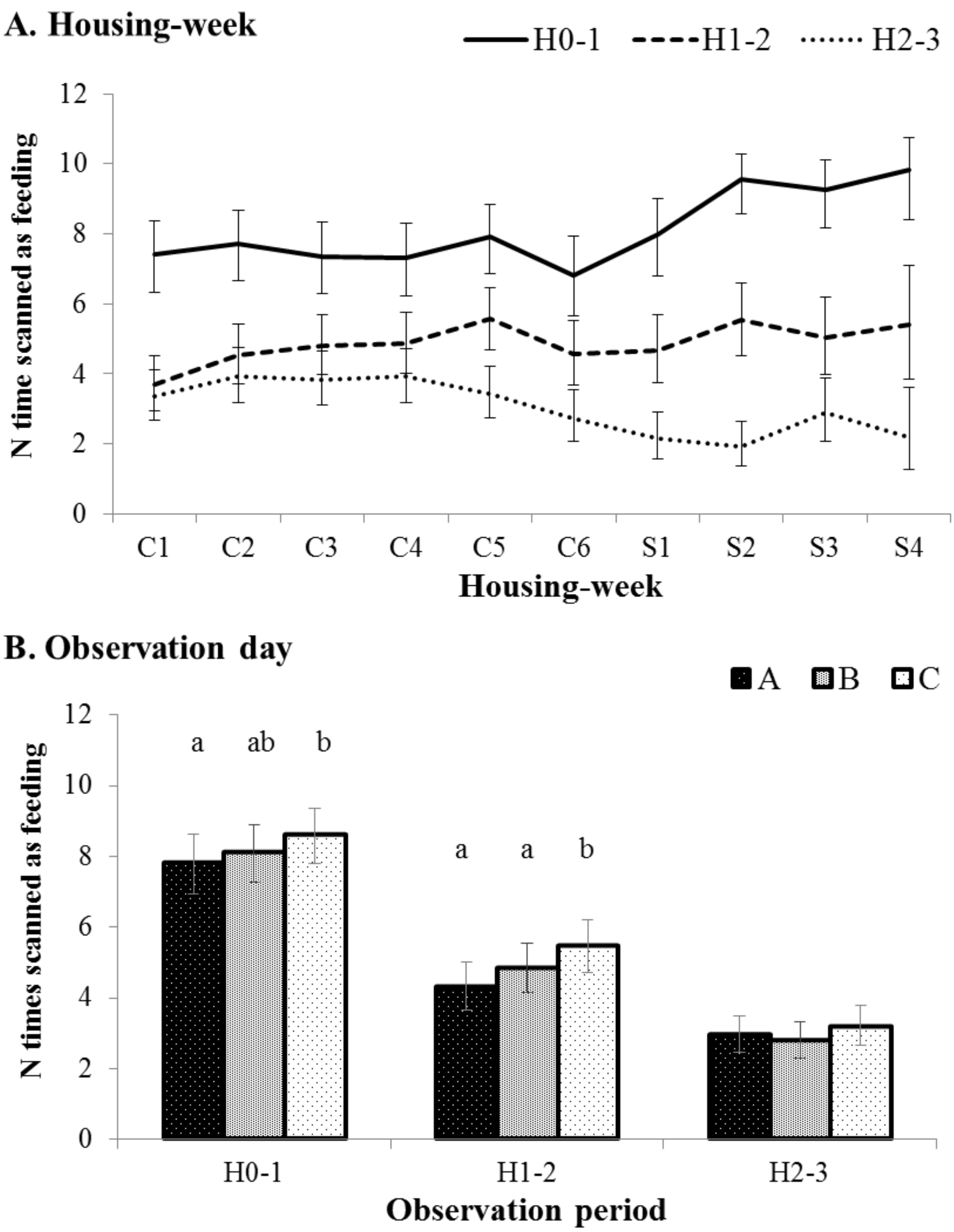

Figure 3.11. Effects of A) week in the cubicle pen (C1-C6) and in the straw yard (S1-S4), and B) observation day in the number of times a cow was scanned as feeding during the H0-1, H1-2 and H2-3 periods. Error bars indicate 95% CIs. Different letters signify differences between observations days at P<0.05.

3.3.5.4. Time spent feeding, time spent standing in the feed alley

Treatment had a significant effect on the time spent feeding during the H0-1, H0-2, H0-3 periods (H0-1: $F_{1,56.2}$=33.1, P<0.001, H0-2: $F_{1,55.6}$=16.9, P<0.001, H0-3: $F_{1,55.2}$=5.8, P=0.020). In all of the three periods, L cows spent more time feeding than H cows (H0-1=L: 47.92±2.4, H: 29.2±2.3 min; H0-2=L: 74.2±3.9, H: 52.3±3.7 min; H0-3=L: 86.6±4.6, H: 71.6±4.4 min). A significant effect of housing-week was also found in all of the three periods, regardless of the treatment groups (H0-1: $F_{,91137.1}$=3.4, P<0.001; H0-2: $F_{9,1087.1}$=3.8, P<0.001; H0-3: $F_{9,1084.8}$=3.1, P=0.001; **Figure 3.12**). From C1 to C5, cows spent 30-40 min and 50-60 min feeding during the H0-1 and H0-2 periods, respectively. The feeding time gradually increased from C6 to S2, resulting in a significant difference in the feeding time during the S2-S4 periods compared to C1-C6 periods. Feeding time during the H0-3 period significantly increased from C1 to C2 (t=2.3, P=0.021) and remained higher until S4, except at C6 and S1.

Observation day also had a significant effect on feeding time in all of the three periods (H0-1: $F_{2,1126.7}$=4.2, P=0.016; H0-2: $F_{2,1078.1}$=11.1, P<0.001; H0-3: $F_{2,11.3}$=11.3, P<0.001). Cows spent more time feeding on observation day C than on observation day A and B in the H0-1 (A: 36.9±1.8, B: 38.3±1.8, C: 40.5±1.8 min), the H0-2 (A: 58.5±3.0, B: 63.0±3.0, C: 68.2±3.0 min) and the H0-3 (A: 74.3±3.5, B: 77.8±3.5, C: 85.1±3.5 min) periods.

A significant effect of lameness score on the time spent feeding was only found in the H0-1 period (non-lame: 40.9±1.7, lame: 36.2±2.2 min, $F_{1,458.6}$=4.9, P=0.028). There was no effect of parity on the time spent feeding in the H0-1 period ($F_{1.45.6}$=2.1, P0.153), but primiparous cows spent a significantly longer time feeding than multiparous cows in the H0-2 period (P: 68.7±4.2, M: 57.8±3.4 min, $F_{1,45.5}$=4.4, P=0.041). The effect of parity disappeared in the H0-3 period (P: 84.9±4.9, M: 73.3±4.0 min, $F_{1,45.5}$=3.4, P=0.066). A significant effect of group size on feeding time was found in the H0-1 ($F_{1,1170.7}$=13.4, P<0.001) and H0-2 ($F_{1,1121.8}$=10.9, P<0.001) periods, where larger group size resulted in cows feeding for a long time (H0-1: +0.7±0.2 min per unit increase, H0-2: +1.1±0.3 min per unit increase).

The time spent standing in the feed alley in the 3h after feed delivery was significantly affected by treatment (H: 5.3 min [4.3, 6.5], L: 2.3 min [1.8, 3.0], $F_{1,79.3}$=39.3, P<0.001), but a significant interaction between treatment and housing-week was also found ($F_{9,1088.0}$=2.9, P=0.003). Cows in the H group stood in the feed alley for significantly longer than the L group from C1 to C4 and S1 to S3 (**Figure 3.13**). The time spent standing in the alley significantly increased from C6 to S1 in the H group, resulting in greater treatment differences during the weeks in the straw yard. There were no significant effects of observation day ($F_{2,1069.5}$=1.8, P=0.161), parity ($F_{1,44.9}$=2.2, P=0.144), lameness score $F_{1,121.2}$=0.3, P=0.564) or group size ($F_{1,793.7}$=3.8, P=0.052) on the time standing in the feed alley.

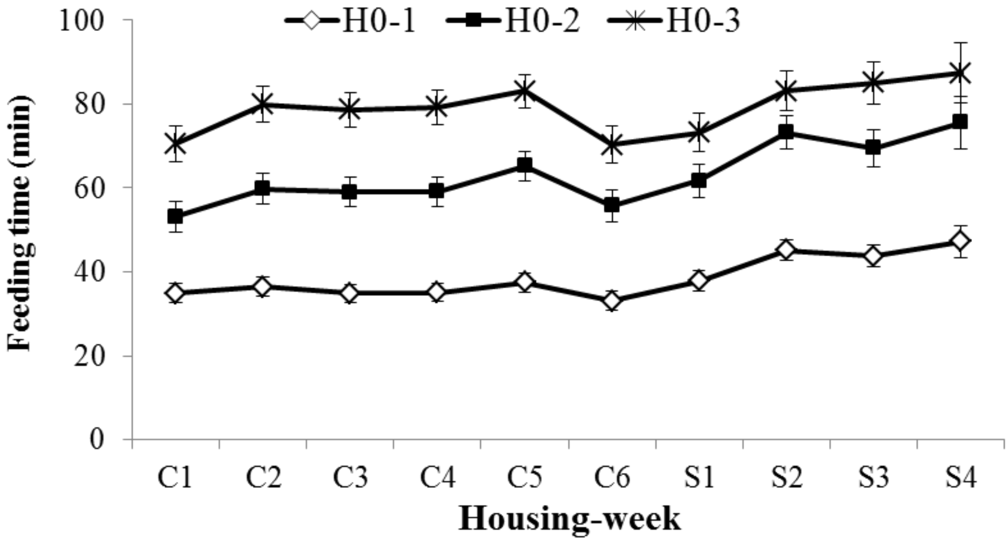

Figure 3.12. Weekly feeding times (±SEM) during the first 1 hour (H0-1), 2 hours (H0-2) and 3 hours (H0-3) after feed delivery in the cubicle pen (C1-C6) and the straw yard (S1-S4).

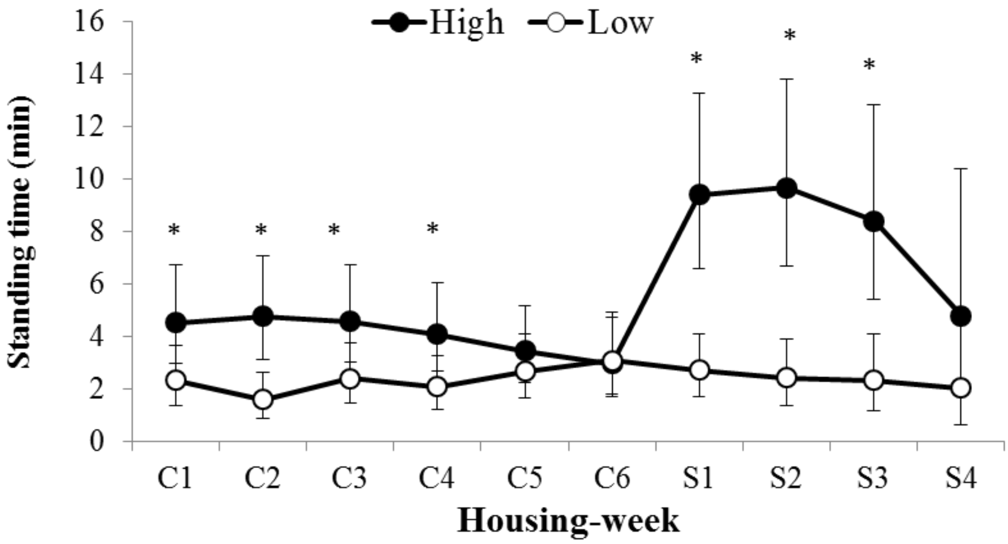

Figure 3.13. Changes over week in the time spent standing in the feed alley (95% CIs) during the 3 hours after feed delivery in the cubicle pen (C1-C6) and the straw yard (S1-S4). Asterisks signify treatment differences at P<0.05.

3.3.6. Social interactions at feed-face

The final models used to analyse the number of each of the social interactions at the feed-face are shown in **Table 3.8**.

Table 3.8. Summary of the final models used for the analyses of social interactions at the feed-face.

Final models for social interactions (Group comparison)	
Agonistic actions	*treatment + observation + housing + group size*
Displacements	*treatment + treatment.housing + housing + observation + group size*
Non-aggressive interactions	*treatment + treatment.housing + housing + observation + group size*
Final models for social interactions (Individual cow competitive behaviour)	
Interactions	
total, as an actor	*treatment + housing-week + observation day + parity + lameness score + group size*
as a recipient	*treatment + treatment.housing-week + housing-week + observation day + parity + lameness score + group size*
Displacements	
as an actor	*treatment + housing-week + observation day + parity + lameness score + group size*
as a recipient	*treatment + housing-week + observation day + parity + lameness score + group size*
Active responses	
Given	*treatment + treatment.housing-week + housing-week + observation day + parity + lameness score + group size*
Received	*treatment + housing-week + observation day + parity + lameness score + group size*

3.3.6.1. Group comparison

Table 3.9 summarises the number of social interactions observed at the feed-face in each of the treatment groups during the observation periods. More agonistic actions were observed in the H group compared with the L group (H: 3.45/group [2.92, 4.05], L: 1.93/group [1.57, 2.34], $F_{1,79.7}$=17.4, P<0.001). Housing type also significantly affected the number agonistic interactions ($F_{1,158.6}$=24.3, P<0.001), which were more frequent in the post-and-rail system (3.73/group [3.11, 4.45]) compared to the yoke system (1.75/group [1.42, 2.13]). The number of displacements was also greater in H group than in L group (H: 0.58 [0.50, 0.68], L: 0.30 [0.23, 0.37], $F_{1,81.5}$=27.1, P<0.001) and was greater in the post-and-rail system than in the yoke system (post-and-rail: 0.63/group [0.52, 0.74], yoke: 0.26/group, [0.19, 0.34], $F_{1,165.6}$=11.5, P<0.001).

However, there was a significant interaction between treatment and housing type on the number of displacements ($F_{1,159.4}$=17.6, P<0.001). The number of displacements observed in the yoke system was not significantly different between treatments, but a significantly greater number of displacements was observed in the post-and-rail system in the H group than in the L group (t=6.8, P<0.001, **Table 3.9**). The number of displacements in the post-and-rail system was significantly greater than the yoke system in the H group (t=5.5, P=0.001), but no difference in the housing type was observed in the L group (**Table 3.9**). A significant effect of observation day was found on the number of displacements ($F_{1,130.8}$=9.4, P=0.003, **Table 3.9**), with a greater number of displacements on observation day C than on observation day A. The number of actions was not different between observation day A and C. The number of actions decreased by 0.01±0.002 times for each unit increase in group size (actions: $F_{1,173.9}$=8.7, P=0.004), whilst the number of displacements was not affected by group size.

There was no significant effect of treatment ($F_{1,79.7}$<0.1, P=0.979), observation day ($F_{1,126.9}$=0.1, P=0.818) and housing type ($F_{1,158.6}$=2.2, P=0.139) on the occurrence of non-aggressive interactions. However, a significant interaction was found between treatment and housing ($F_{1,155.2}$=9.8, P=0.002, **Table 3.9**). There was a significantly greater probability of cows experiencing non-aggressive interactions in the post-and-rail feed-face than in the yoke feed-face in the H group (t=3.2, P=0.001). No effect of housing type was found in the occurrence of non-aggressive interactions in the L group. Non-aggressive interactions were more likely to occur with increasing group size (probability: increase by 0.04±0.05 per unit increase in group size, $F_{1,173.9}$=4.2, P=0.040).

Table 3.9. The number of actions, displacements and non-aggressive interactions observed in each of the treatment groups during the observation period (0-20min, 40-60min, 80-100min after feed delivery). The figures in the table show predicted means for the number of interactions/group [95% CIs].

NA: not applicable

	Treatment and Housing type				**Observation day**		**P value**			
	High stocking		Low stocking		A	C	treatment	housing	observation day	treatment.housing
	Yoke	Post-rail	Yoke	Post-rail						
Actions	2.00 [1.39, 2.80]	5.45 [4.41, 6.69]	1.36 [1.04, 1.72]	2.81 [2.13, 3.64]	2.45 [2.11, 2.83]	2.82 [2.44, 3.24]	**<0.001**	**<0.001**	0.152	0.062
Displacements	0.29 [0.17, 0.43]	0.93 [0.78, 1.08]	0.23 [0.16, 0.31]	0.37 [0.26, 0.50]	0.37 [0.31, 0.43]	0.50 [0.43, 0.57]	**<0.001**	**<0.001**	**0.003**	**<0.001**
Non-aggressive interactions	0.29 [0.13, 0.53]	0.83 [0.68, 0.92]	0.60 [0.46, 0.74]	0.67 [0.46, 0.82]	0.62 [0.51, 0.72]	0.61 [0.49, 0.71]	0.979	0.139	0.870	**0.002**

3.3.6.2. Individual cow competitive behaviour

Table 3.10 summarises the number of aggressive interactions that each cow was involved in during the observation period.

Number of interactions

A significant effect of treatment was found in the total number of interactions a cow was involved in ($F_{1,77.1}$=17.6, P<0.001), the number of interactions a cow was involved in as an actor ($F_{1,72.1}$=14.1, P<0.001) and the number of interactions a cow was involved in as a recipient ($F_{1,77.6}$=18.7, P<0.001). Greater numbers of total interactions, interactions as an actor and as a recipient were observed in the H group than in the L group (**Table 3.10**). There was a significant effect of housing-week on the total number of interactions a cow was involved in ($F_{9,760.6}$=3.9, P<0.001; **Figure 3.14A**), the number of interactions a cow was involved as an actor ($F_{9,757.9}$=2.1, P=0.029; **Figure 3.14B**), and the number of interactions a cow was involved as a recipient ($F_{9,760.8}$=2.5, P=0.007; **Figure 3.14C**). The number of interactions (total, as an actor and a recipient) were significantly greater (P<0.05) during the first three weeks in the straw yard compared to the weeks in the cubicle pen. There was a significant interaction between treatment and housing-week in the number of interactions as a recipient ($F_{9,748.0}$=2.4, P=0.011). Cows in the H group received a significantly greater number of interactions in S1, S2 and S3 compared to the weeks in the cubicle pen (except C6), whilst a significant increase was only observed at S1 in the L group. This resulted in a significant treatment differences at C6, S1 and S2.

A significant effect of parity was found in the total number of interactions a cow was involved in ($F_{1,44.8}$=14.8, P<0.001), the number of interactions as an actor ($F_{1,45.3}$=5.5, P=0.024) and as a recipient ($F_{1,45.1}$=15.5, P<0.001). Primiparous cows were more often involved in aggressive interactions than multiparous cows (**Table 3.10**). There was no effect of lameness score on the number of interactions that a cow was involved in as an actor, but non-lame cows were more likely to be a recipient of an interaction than lame cows ($F_{1,132.7}$=4.0, P=0.017, **Table 3.10**). The total number of interactions was also greater among non-lame cows than lame cows ($F_{1,139.5}$=6.5, P=0.012). A significant effect of group size was found on the total number of interactions, interactions as an actor and a recipient, where the numbers of interactions decreased as group size increased (total interactions: $F_{1,706.8}$=14.9, P<0.001; interactions as an actor: $F_{1,752.8}$=17.7, P<0.001; interactions as a recipient: $F_{1,637.2}$=4.0, P=0.047). No significant effect of observation day was found on any of the types of interactions measured (**Table 3.10**).

Table 3.10. The number of aggressive interactions a cow was involved in during the observation period (0-20min, 40-60min, 80-100min after feed delivery). The figures in the table show predicted means for the number of interactions per cow [95% CIs].

	Treatment		**Observation day**		**P value**	
	High stocking	Low stocking	A	C	Treatment	Observation day
Total interactions	4.86 [4.03, 5.82]	2.58 [2.02, 3.25]	3.35 [2.84, 3.93]	3.82 [3.23, 4.50]	**<0.001**	0.092
Interactions as an actor	2.29 [1.80, 2.86]	1.08 [0.74, 1.48]	1.49 [1.19, 1.84]	1.74 [1.40, 2.14]	**<0.001**	0.098
Interactions as a recipient	2.27 [1.84, 2.77]	1.31 [0.98, 1.70]	1.68 [1.51, 2.00]	1.83 [1.51, 2.18]	**<0.001**	0.326
Displacements as an actor	0.75 [0.56, 0.97]	0.45 [0.28, 0.64]	0.54 [0.40, 0.69]	0.65 [0.50, 0.82]	**0.031**	0.090
Displacements as a recipient	1.13 [0.91, 1.37]	0.60 [0.42,0.80]	0.82 [0.66, 0.98]	0.77 [0.61, 0.94]	**0.006**	0.539
Active response as an actor	1.24 [0.98, 1.54]	0.58 [0.39, 0.81]	0.77 [0.61, 0.96]	1.00 [0.80, 1.22]	**<0.001**	**0.014**
Active response as a recipient	1.35 [1.07, 1.67]	0.45 [0.26, 0.67]	0.78 [0.60, 0.97]	0.92 [0.72, 1.14]	**<0.001**	0.143

Table 3.10. continued.

	Parity		Lameness score		P value	
	Multiparous	Primiparous	Non-lame	Lame	Parity	Lameness score
Total interactions	2.76 [2.26, 3.32]	4.58 [3.75, 5.57]	4.19 [3.61, 4.83]	3.04 [2.40, 3.82]	**<0.001**	**0.012**
Interactions as an actor	1.30 [0.98, 1.67]	1.98 [1.50, 2.54]	1.85 [1.52, 2.23]	1.40 [1.00, 1.87]	**0.024**	0.080
Interactions as a recipient	1.30 [1.02, 1.60]	2.30 [1.85, 2.82]	2.06 [1.76, 2.40]	1.47 [1.11, 1.89]	**<0.001**	**0.017**
Displacements as an actor	0.50 [0.35, 0.66]	0.70 [0.50, 0.92]	0.68 [0.54, 0.84]	0.51 [0.33, 0.71]	0.121	0.112
Displacements as a receiver	0.60 [0.45, 0.76]	1.01 [0.80, 1.25]	0.90 [0.75, 1.06]	0.69 [0.50, 0.91]	**0.002**	0.090
Active response as an actor	0.68 [0.51, 0.88]	1.11 [0.85, 1.39]	1.01 [0.84, 1.21]	0.76 [0.53, 1.02]	**0.007**	0.088
Active response as a recipient	0.66 [0.47, 0.86]	1.06 [0.80, 1.35]	0.92 [0.74, 1.12]	0.77 [0.53, 1.05]	**0.015**	0.333

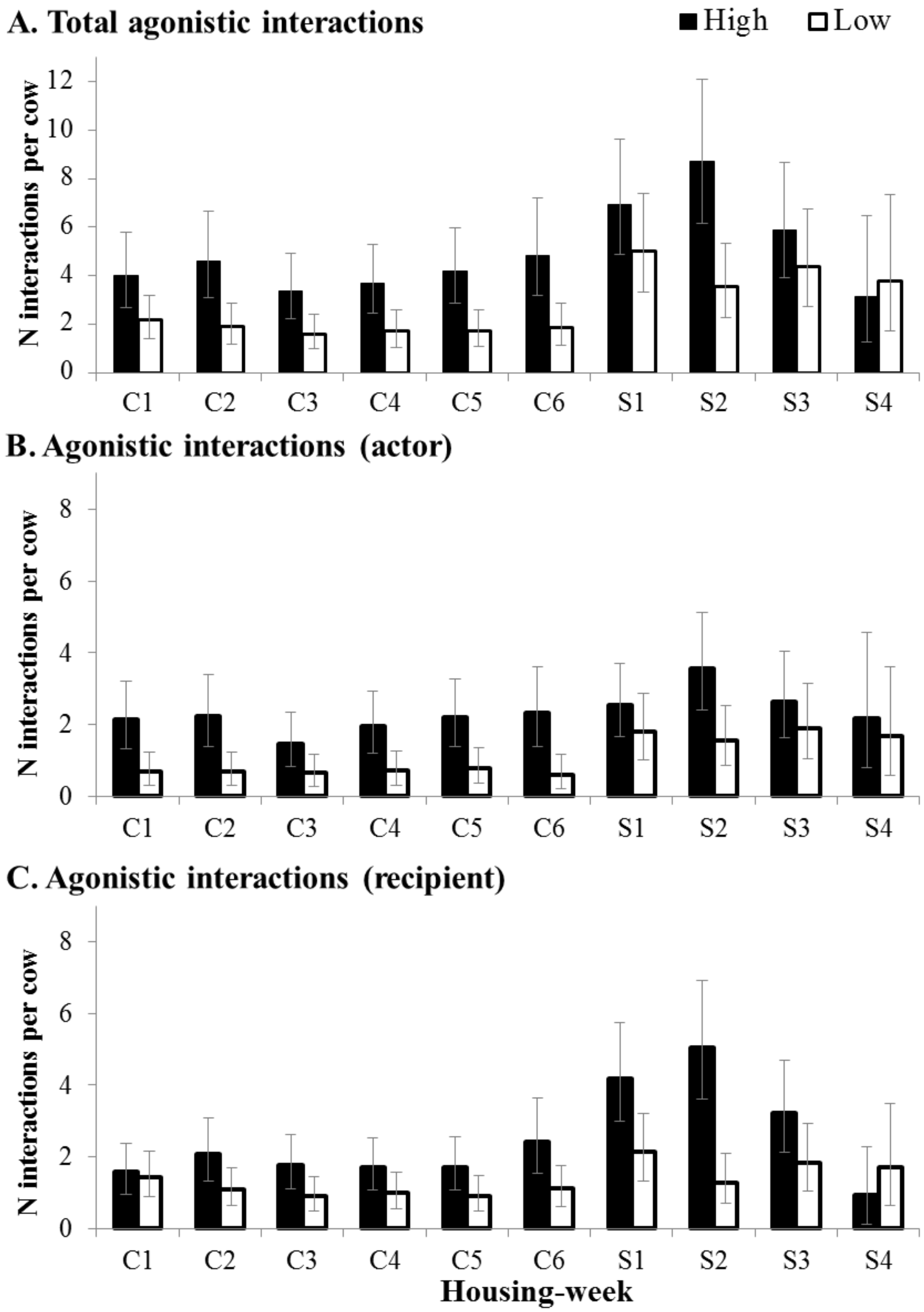

Figure 3.14. The total number of interactions a cow was involved in (A), the number of interactions a cow was involved in as an actor (B) or as a recipient (C) in each of the weeks in the cubicle pen (C1-C6) and the straw yard (S1-S4). Error bars indicate 95% CIs.

Number of displacements

There was a significant effect of treatment on the number of displacements cows expressed as an actor ($F_{1,68.9}$=4.9 P=0.031) and the number of displacements experienced as a recipient ($F_{1,77.7}$=8.0, P=0.006). The number of displacements both as an actor and as a recipient was higher in the H group compared to the L group (**Table 3.10**). A significant effect of housing-week was observed in the number of displacements as a recipient ($F_{9,760.9}$=2.0, P=0.037; **Figure 3.15B**), but not as an actor ($F_{9,756.3}$=1.2, P=0.269; **Figure 3.15A**). Cows in both groups were displaced by other cows significantly more often in S1 compared to C1-C6, and in S2 compared to C3, C4 and C6.

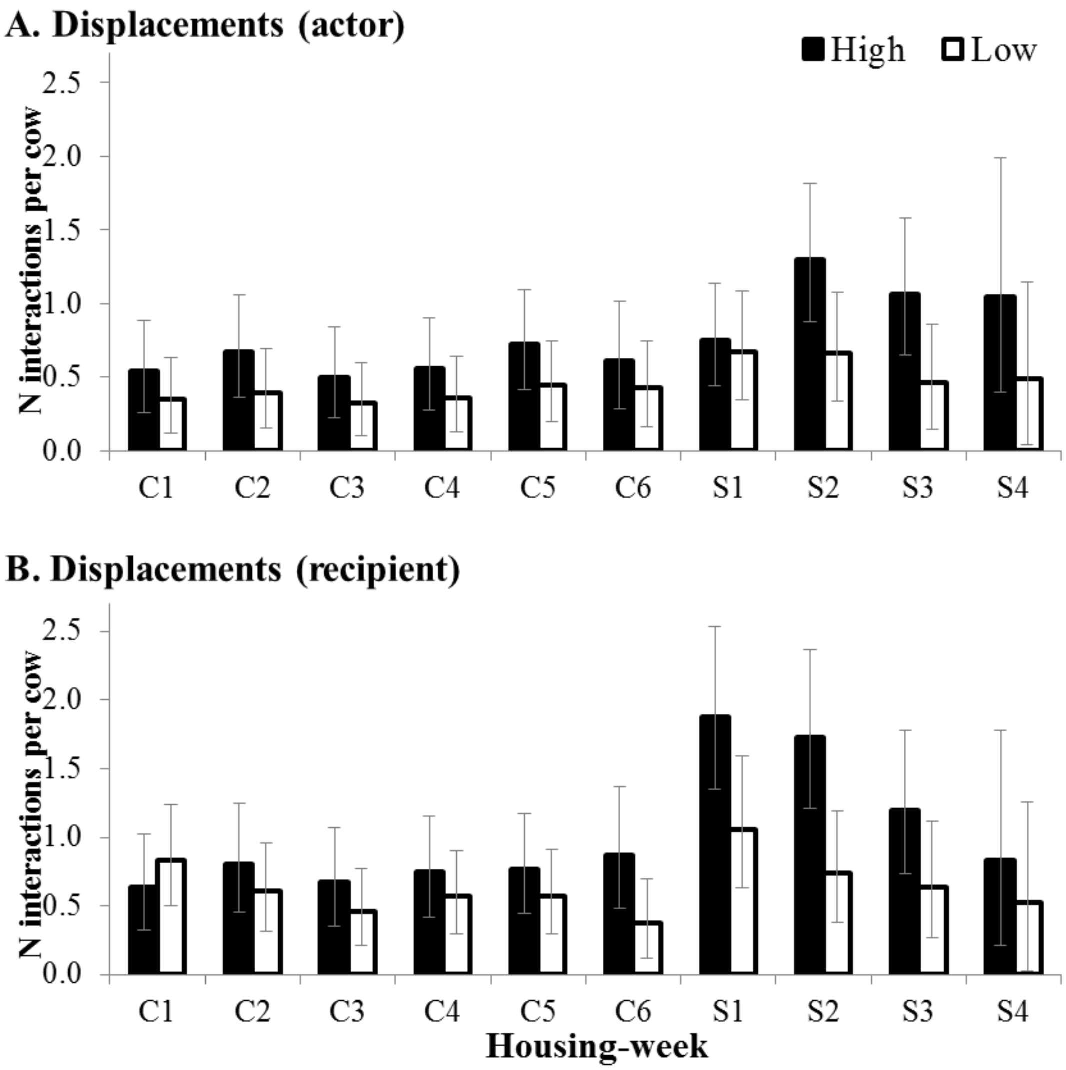

Figure 3.15. The number of displacements a cow was involved as an actor (A) and a recipient (B) in each of the week in the cubicle pen (C1-C6) and the straw yard (S1-S4). Error bars indicate 95% CIs.

The number of displacements as an actor was not different between primiparous and multiparous cows ($F_{1,45.5}$=2.5, P=0.121), but a greater number of displacements as a recipient was observed in primiparous cows than multiparous cows ($F_{1,44.8}$=10.4, P=0.002, **Table 3.10**). The number of displacements both as an actor and as a recipient decreased as group size increased (actor: $F_{1,770.5}$=13.2, P<0.001, recipient: $F_{1,700.5}$=19.2, P<0.001). There were no significant effects of observation day and lameness score on the numbers of displacements as an actor or as a recipient (**Table 3.10**).

The number of active responses gave and received

There was a significant effect of treatment on the number of active responses (fought back) a recipient cow gave ($F_{1,79.4}$=24.6, P<0.001) and an actor cow received ($F_{1,77.6}$=24.7, P<0.001). Cows in the H group gave and received more active responses than cows in the L group (**Table 3.10**). A significant interaction between treatment and housing-week was found in the number of active responses a recipient cow gave ($F_{9,748.5}$=2.5, P=0.008, **Figure 3.16A**). In the H group, the number of active responses a cow gave significantly increased from C5 to C6 (P=0.024) and peaked in S2, whilst a significant increase was only observed in S3 in the L group compared to C3, C4 and C5. Housing-week did not affect the number of active responses an actor cow received ($F_{9,760.9}$=1.1, P=0.400; **Figure 3.16B**).

A significant effect of parity was also found in the number of active responses a recipient cow gave ($F_{1,45.1}$=7.9, P=0.007) and an actor cow received ($F_{1,44.7}$=6.4, P=0.015). In both treatment groups, primiparous cows gave and received active responses more often than multiparous cows (**Table 3.10**). There was a significant effect of observation day on the number of active responses a recipient cow gave ($F_{1,735.2}$=6.0, P=0.014), which showed that cows gave active responses more frequently on observation day C than observation day A (**Table 3.10**). The number of active responses an actor cow received was not significantly different between observation days ($F_{1,743.3}$=2.2, P=0.143). Cows received more active responses when the group size was smaller ($F_{1,699.8}$=11.8, P<0.0001), but the group size did not affect the number of active responses a cow gave ($F_{1,618.8}$=0.6, P=0.457). Lameness score had no significant effect on the number of active responses a cow gave or received.

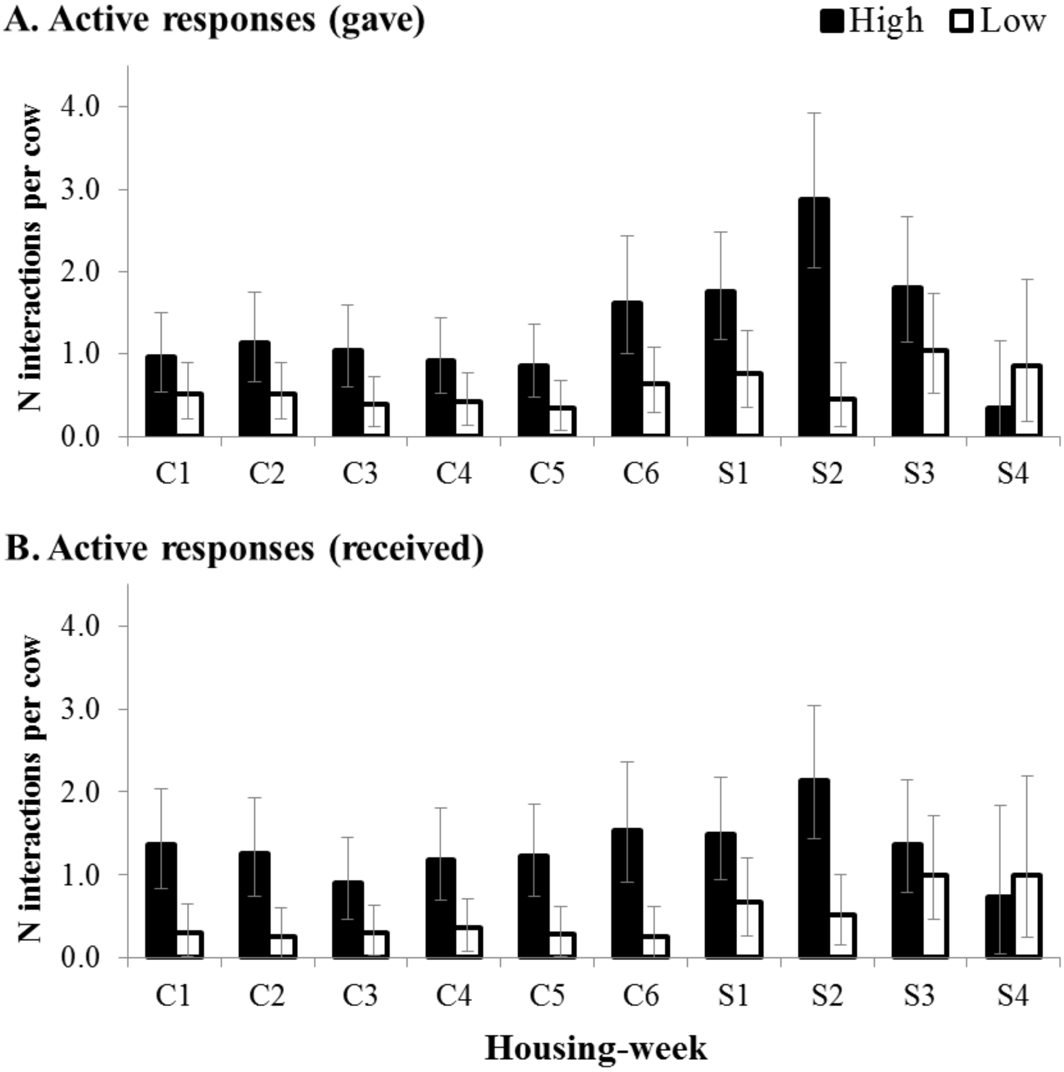

Figure 3.16. The number of active responses a recipient cow gave (A) or an actor cow received (B) in each of the weeks in the cubicle pen (C1-C6) and the straw yard (S1-S4). Error bars indicate 95% CIs.

3.4. Discussion

3.4.1. Feed space occupancy and access to feed

Treatment

High stocking density treatment for dairy cows during the dry period resulted in a constantly crowded feed-face during the first three hours after fresh feed delivery. Cows are most motivated to feed immediately after feed delivery (DeVries and von Keyserlingk, 2005), so that the time within 60-90 min of feed delivery is considered as a peak feeding period (DeVries et al., 2004; Endres et al., 2005; Huzzey et al., 2006). The present study showed that more than 50% of the feed-face was occupied during the peak feeding period (60 min after feed delivery) in both treatment groups. Moreover, overstocking of the feed-face resulted in more than 70% of the feed-face being used during the peak feeding period, and more than 50% of the feed-face still being used after three hours from feed delivery. In contrast, in the low stocking density treatment, where all cows had access to the feed-face at the same time, the feed-face occupancy decreased to less than 50% after one hour from fresh feed delivery and the percentage occupancy continued to decrease to less than 25% at three hours after feed delivery.

The present study found that time spent feeding during one hour after feed delivery was shorter in the overstocked group. Interestingly, the current study found that the feeding time between one and two hours after feed delivery was not affected by stocking density, and the feeding time between two and three hours after feed delivery was longer when overstocked. However, this did not affect the total feeding time during the first three hours after feed delivery, and overstocking still reduced the feeding time by 22 min during the first two hours and by 15 min during the first three hours after feed delivery. This result was similar to previous findings, where DeVries et al. (2004) reported that cows reduced their feeding time when the feeding space was reduced from 1.0 to 0.5m per cow. Moreover, the change in feeding time was particularly prominent during the peak feeding period (the first 90 minutes after feed delivery; DeVries et al., 2004). Huzzey et al. (2012) also reported that overstocking of the feed-face and lying areas (0.34m vs 0.67m feed-face space per cow and 0.5 vs 1.0 cubicle per cow) reduced the proportion of feeding time during the three hours after feed delivery.

The current study found that cows were less likely to approach the feed-face and to start feeding within five minutes after feed delivery when overstocked. Huzzey et al. (2012) also reported an extended time for dry cows to approach the feed-face after delivery of fresh feed when overstocked. The lower probability of cows starting to feed within five min after feed delivery resulted in a reduced likelihood of cows in the overstocked group having a 60-min meal within the three hours after feed delivery. In the high stocking group, the percentage of cows observed at the feed-face was increased after the two hours from feed delivery. Even though cows increased their feeding activity after the two hours following feed delivery, overstocking appeared to disturb the feeding activity of the cows during the three hours after feed delivery.

Parity

The current study did not find a significant parity effect on the probabilities of approaching the feed-face and starting to feed. However, the current study showed that primiparous cows were more likely to have a longer feeding time during the first two hours after feed delivery and to achieve a 60-min meal within three hours after feed delivery, irrespective of the stocking density. Huzzey et al. (2012) found that the extended time taken for cows to approach the feed-face during the overstocked period was mainly attributable to primiparous cows, as overstocking did not change the time taken for multiparous cows to approach the feed-face. Similarly, a study by Lobeck-Luchterhand et al. (2015) compared 100% feed-face stocking density with 80% and reported that nulliparous Jersey cows reduced their feeding time while parous Jersey cows (parity≥1) increased their feeding time with improved feed access. The primiparous cows in the current study had never experienced a dry period, but they had already experienced the whole lactation period with multiparous cows. Therefore, a comparison with other studies is difficult. However, the current study suggests that primiparous cows with an experience of the whole lactation period were more successful at coping with overstocking than multiparous cows, in terms of feeding behaviour.

Housing/feed-face type and changes over week

The current study found higher feed-face occupancy in the post-and-rail system during the first hour after feed delivery compared with the yoke system when overstocked. This is similar to Huzzey et al. (2006), who reported that more cows were observed at the post-and-rail feed-face than the headlock (or yoke) feed-face within 60 minutes after feed delivery. In addition, the current study found that the probability of cows approaching the feeding area and starting to feed within five minutes after feed delivery was higher during the weeks in the straw yard (i.e. when post-and-rail system was used) compared to most of the weeks in the cubicle pen (i.e. when the yoke system was used). Moreover, cows were more likely to be observed feeding within 60 minutes after feed delivery during the same periods.

The feed-face occupancy at its maximum exceeded 100% during the first ten minutes after feed delivery when the post-and-rail system was used. This means that the feed-face space during this period was less than 0.30 m per cow. DeVries et al. (2004) reported that cows decreased the distance to neighbouring cows when the feed space was reduced, which is only possible in the post-and-rail system where there is no partition between neighbouring cows. Together with a higher motivation to feed immediately after feed delivery, easier access to the post-and-rail feed-face may have allowed more cows to feed at the same time.

It has been reported that a post-and-rail system increased the daily feeding time (Huzzey et al., 2006) and feeding time during the peak feeding period (Endres et al., 2005) compared with a yoke system. The current study found that cows had longer feeding time during the first two hours after feed delivery during the weeks 2 to 4 in the straw yard, compared to the weeks 2 and 6 in the cubicle pen. However, this effect disappeared during the three hours after feed delivery.

Easier access to the feed-face may have helped cows achieve 60 min-meal within three hours after feed delivery. However, the probability of cows achieving 60-min meal within 60 minutes after feed delivery remained lower in the H group throughout the treatment period and the first week in the straw yard was the lowest. This may suggest that easier access to the feed-face may have led to an increase in competition during the peak feeding time in the H group, resulting in the failure of many cows to reach the average meal duration during the weeks in the straw yard.

Increased feeding activity during the weeks in the straw yard may be explained by a higher motivation for feed derived from the increasing energy requirements during the transition period (Jouany, 2006). In contrast, previous studies have shown that cows reduce dry matter intake before calving (Bertics et al., 1992; Ingvartsen et al., 1992; Grant and Albright, 1995; Grummer, 1995) because the growing fetus reduces the rumen volume and thus feed intake. The current study did not measure dry matter intake, and so it is not possible to look for any association between the extended feeding time and feed intake.

Over the weeks in the cubicle pen, cows gradually decreased their probability of approaching the feeding area and the probability of starting to feed within five minutes after feed delivery. However, from the second week, cows were more likely to achieve 60 min-meal within the two hours after feed delivery and were more often observed feeding between one and two hours after feed delivery. The probabilities further increased in week4 and week5 compared to week 1. This may indicate that cows gradually adapted to a new environment (dry group) and were better able to ensure the average feeding time during the peak feeding period even though they did not access to the feeding area immediately after feed delivery.

Lameness

The current study did not find any evidence that a reduced stocking density may be beneficial for lame cows. Lame cows in the low stocking group had a lower probability of starting to feed immediately after feed delivery compared to non-lame cows. This is probably because the low stocking density theoretically allowed all cows to feed at the same time, which reduced competition for access to the feed-face, and so lame cows took more time to approach the feed-face. The limited feed space in the high stocking group imposed the same restriction on access to the feed-face for all of the cows, and having lameness may not have been the only reason to delay the time to start feeding after feed delivery.

Studies on the effect of stocking density on the feeding behaviour of lame cows are scarce. However, previous studies (González et al., 2008; Gomez and Cook, 2010) have shown the impact of lameness on the daily feeding activity of lactating cows. In these studies, lame cows had a lower daily feed intake, a shorter daily feeding time and fewer daily feeder visits compared with non-lame cows. The current study only measured feeding activities during the peak feeding period, and found no effect of lameness on feeding time during the first three hours after feed delivery. However, a lower probability of lame cows coming to the feed-face immediately after the delivery of feed resulted in a shorter feeding time in the first one hour, and a lower probability of reaching a 60-min meal within the peak feeding period.

Days from mixing

Cows spent longer feeding and more cows were present at the feed-face on the sixth day after weekly regrouping, compared with the first day after regrouping. Additionally, the feed-face on the third day after weekly regrouping was more crowded than on the first day after regrouping during the first one hour after feed delivery. It is possible that cows had gained more confidence in accessing the feed-face by the sixth day, compared with the first day after regrouping. This may also explain why the probability of cows approaching the feed-face and starting to feed after feed delivery increased on the sixth day compared with the first day after regrouping. It has been reported that cows become more vigilant in novel environments and in the presence of potential threats, affecting feeding behaviour (Welp et al., 2004). Therefore, cows may be more vigilant in accessing the feed-face on the first day after regrouping, when they undertake their first feed with new group members in an unstable social environment.

Weekly regrouping makes it difficult to pinpoint when social stabilisation had occurred or would occur. The results from the current study would indicate that cows were more confident in accessing the feed-face and engaging in feeding activity on the sixth day after regrouping than the first day after regrouping. At the same time, it is also possible to argue that cows are still in the process of social stabilisation within the group on the sixth day.

Time standing inactive

Similar to previous studies (Olofsson, 1999; Huzzey et al., 2006; Proudfoot et al., 2009), the current study found that increased stocking density or increased competition at the feed-face resulted in longer durations of time spent standing inactive in the feeding area. Extended standing time is associated with a higher risk of leg and hoof lesions in lactating cows especially on a hard floor surface (Leonard et al., 1996; Galindo and Broom, 2000). The current study also found that the time spent standing inactive in the feeding alley was longer in the post-and-rail system than in the yoke system. This is probably due to the design of the pen, where the straw yards had a more flexible access to the feed-face and a wider space allowance in the feeding alley compared to the cubicle pens. It is worth noting that Huzzey et al. (2006) reported the opposite results to this study. They did not discuss why there was a difference between the types of feed barriers in their study, but the different outcomes from these two studies would suggest that standing time in the feeding alley might be affected by space allowance therein, rather than feed-face type.

3.4.2. Social interactions

Treatment

The current study observed aggressive interactions at the feed-face in each of the treatment groups from 0-20 min, 40-60 min and 80-100 min after feed delivery, which aimed to capture as many interactions during the peak feeding period as possible. The current study found that overstocking resulted in more aggressive interactions, confirming previous findings (Olofsson, 1999; DeVries et al., 2004; Huzzey et al., 2006; Proudfoot et al., 2009; Krawczel et al., 2012a). The current study also found that such aggressive behaviours were observed at low stocking densities, which is in agreement with previous studies that reported that cows were involved in aggressive interactions at the feed-face when the stocking density was 100% or less (1.0 or 1.3 yoke/cow: Huzzey et al., 2006; Lobeck-Luchterhand et al., 2015). Non-aggressive interactions normally occurred when a cow was pushed aside/away by a neighbouring cow that received aggressive actions, and it was hypothesised that feeding patterns of cows in the high stocking group would be more often disturbed by both aggressive and non-aggressive interactions. However, the current study found that the stocking density did not affect the number of those non-aggressive interactions.

In addition to the number of interactions per group, the current study also investigated the number of aggressive interactions a cow was involved in. Cows were more likely to initiate and receive aggressive behaviours or displacements from the feed-face at the high stocking density. Moreover, cows at the high stocking density fought back more often when another cow initiated the aggressive interaction, and were often unsuccessful in their attempt to displace other cows. DeVries et al. (2004) suggested that increased competition at a narrower feed-face during the peak feeding period could result in a decreased feeding time during this period. The current study also suggested that overstocking forced cows to interact with other cows rather than feeding, reducing the time available for cows to engage in feeding activity.

Parity

Overstocking increased the number of aggressive interactions in both primiparous and multiparous cows. However, primiparous cows were more often involved in aggressive interactions than multiparous cows, regardless of the stocking density. Primiparous cows were more likely to be displaced by other cows compared to multiparous cows, although they fought back more. Primiparous cows were also more likely to receive active responses compared to multiparous cows, which may explain why there was no parity difference in the number of successful displacement attempts. Proudfoot et al. (2009) found a similar outcome, in that both primiparous cows and multiparous cows increased the frequency of displacing other cows at high stocking density, but only primiparous cows were more frequently displaced with an increased stocking density.

The primiparous cows in the current study had experienced the dry period for the first time, which involved weekly regrouping and a change in housing type in the middle of the dry period. Research on cows and calves has shown that previous social experiences can help individuals better adapt to a challenging social environment such as overstocking and regrouping (Bouissou, 1980; Veissier et al., 2001; Boyle et al., 2013). Therefore, the results from the current study would suggest that multiparous cows were better at adjusting to a dynamic social environment (more typical to dry cows), by reducing their feeding time during the peak feeding period and avoiding competition. Indeed, the current study found that primiparous cows had a longer feeding time than multiparous cows during the first two hours after feed delivery.

Housing/feed-face type and changes over week

Previous research has shown that a feed-face with partitions between neighbouring cows can reduce competitive behaviour at the feed-face (cows: Endres et al., 2005; Huzzey et al., 2006; calves: Jensen et al., 2008). The current study found that competitive behaviours per group were more frequently observed at the post-and-rail system compared with the yoke system, regardless of the stocking density. The number of displacements and non-aggressive interactions observed was more frequent in the post-and-rail system than the yoke system when overstocked, and the effect of feed barrier type was not observed in the low stocking density group. Therefore, the effect of feed barrier design could be more important under high stocking conditions.

At the individual level, the frequency of aggressive interactions was higher during the weeks when the post-and-rail system was used, compared to the weeks when the yoke system was used. The current study also found that the effect of stocking density on competitive behaviours at the feed-face was much more distinct during the weeks in the straw yard compared to the cubicle pen. For example, cows in the high stocking density treatment received aggression from other cows and fought back more frequently during the weeks in the straw yard compared to the weeks in the cubicle pen, whereas this effect was observed only in a single week in the L group. These results may also suggest that the effect of overstocking on competition at the feed-face could be exacerbated by using a post-and-rail feed barrier design.

A previous study on Jersey cows (Lobeck-Luchterhand et al., 2014) investigated the effect of weekly regrouping on the displacement rate for five weeks during the close-up period, and found that the greatest rate of displacement occurred during the first week after entering the treatment group, compared with the rest of the weeks. However, the current study found no detectable changes over the first five weeks in the treatment group. The discrepancy in the change from the first weeks in the treatment period may be because the study on Jersey cows included the behavioural data from the day new cows entered the group. In contrast, the current study conducted behavioural observations one day after new cows were introduced. Although no fresh feed was delivered between the entry of new cows to the dry group and the first behavioural observations, newly introduced cows might have already acclimatised to a new environment and were less likely to be involved in agonistic interactions.

Lameness

Lame cows were less likely to receive aggressive interactions compared with non-lame cows, which resulted in a greater number of total interactions in non-lame cows. However, the lameness score did not affect the number of displacements or aggressive responses. Although non-lame cows received aggressive interactions more frequently, this did not affect their feeding time during the peak feeding period. For lame cows, it may be painful to walk to the feed-face, and so they may want to reduce the frequency of visits to feed by staying and feeding longer at the feed-face.

Days after mixing

The current study found that the incidence of displacements per group were higher on the sixth day after weekly regrouping than on the first day after weekly regrouping. This contradicts the initial hypothesis that agonistic social interactions would have declined by the sixth day compared with the first day. Previously, Hasegawa et al. (1997) reported that changes in social behaviour after regrouping remained for two weeks. Kondo and Hurnik (1990) also reported that physical interactions dominated agonistic social behaviour after regrouping, but non-physical interactions became more predominant once social stability was established. In their study, it took 2-4 days for group stabilisation to occur if heifers had no previous experience of regrouping, whilst heifers with previous experience of regrouping took less than two days to stabilise within the group. Similarly, a study on lactating cows showed that agonistic social interactions peaked on the day of mixing, but declined to baseline levels after two days (von Keyserlingk et al., 2008).

At the individual animal level, the number of aggressive interactions a cow was involved in was not different between the first and sixth day after regrouping, although cows were more likely to fight back against aggression from other cows on the sixth day. No notable effect of observation day is probably due to a larger variation in the number of interactions a cow was involved in on the first day after regrouping. The increase in the number of displacements at the group level on the sixth day after regrouping was probably associated with the higher feed-face occupancy and the higher percentage of cows at the feed-face in the three hours after feed delivery on the sixth day after weekly regrouping. Indeed, the number of yokes and feed space available per cow was the lowest on the sixth day after weekly regrouping in the first two hours after feed delivery. Reduced inter-cow distance has been associated with increased social interactions (DeVries et al., 2004).

The current study analysed physical and non-physical interactions together, and did not distinguish between the behaviour of newly introduced cows and resident cows. This may be one of the reasons for the different outcomes compared to previous studies. The results from the current study suggest that cows engaged less in feeding activity during the peak feeding period on the first day after regrouping, resulting in fewer competitive behaviours at the feed-face compared with the sixth day after regrouping. It would be beneficial to study how long it takes to resolve the effect of regrouping on feeding and social behaviour at the feed-face, but this would have been confounded by the weekly re-grouping of cows on the farm.

3.4.3. Group size

The current study showed that cows were more likely to start feeding within 5 minutes after feed delivery with a larger group size. The current study also found that competition at the feed-face decreased and feeding time during the first 2 hours after feed delivery increased when the group size was larger. In contrast, Telezhenko et al. (2012) found no effect of group size on the number of displacements at the feeder. They observed competitive behaviour at the feed-face seven days after regrouping, and every treatment group had the same feed-face length of 0.6m per cow. However, they also found that cows' activity increased in larger pens regardless of stocking density. In the current study, the feed-face and the lying area were adjusted according to the number of cows in each of the treatment groups, which resulted in a wider feeding area and larger pen size with increasing group size. The wider feeding space may have encouraged cows to come to the feed-face. These results suggest that the feed-face length in addition to the stocking density could affect feeding behaviour.

3.4.4. Locomotor activity

Treatment

The current study found no overall treatment effect on daily lying bouts and daily lying proportions. The current study restricted access to the feed-face, but each cow had at least one cubicle and 6.0 m^2 of lying space, ensuring all cows could lie down at the same time. In contrast, it has been previously reported that a decreased space allowance of lying area (100-150%: Fregonesi et al., 2007a) or both lying and feeding areas (100%-142%: Hill et al., 2009; Krawczel et al., 2012b) resulted in a decreased lying time. Previous studies have also shown that overstocking of the feeding area alone did not affect the daily lying time in dry cows (Huzzey et al., 2006) and lactating cows (Collings et al., 2011). Both of these studies also provided one cubicle per cow for both overstocked and control groups.

Telezhenko et al. (2012) reported that cows reduced their lying time when the availability of cubicles was reduced from multiple cubicles to a single cubicle per cow, but their daily lying time was never below the recommended level. Munksgaard et al. (2005) reported that cows prioritised time for lying over feeding when both lying and feeding space were restricted. Therefore, cows' lying behaviour may not be compromised if each cow has at least one cubicle or lying space. In contrast, Fustini et al. (2017) found no difference in daily lying time when cows were housed on a "bedded pack" with 7.8m^2 of lying space per cow compared with 3.3m^2. A study on lactating cows also found no difference in lying time when comparing 9.0m^2 and 4.5m^2 of straw-bedded space (Fregonesi and Leaver, 2002). However, Fustini et al. (2017) found that daily lying time in both stocking densities did not reach the recommended daily lying time (12 hours/day, Munksgaard et al., 2005), suggesting that cows' motivation for lying was not satisfied with this restricted lying space.

Housing type and changes over week

Fustini et al. (2017) suggested that an increase in the mean number of lying bouts in the first week of overstocking may indicate an adjustment period to the higher stocking density. However, the current study did not find any significant treatment effect on the activity level in the first week of the treatment (C1). The current study suggests that cows were less active and less likely to lie down during the first week of the dry period, with no significant difference between treatments. However, levels of increase in MotionIndex and step count were more prominent in the H group. Fustini et al. (2017) found that cows took a greater number of steps per hour during the three weeks before parturition at a high stocking density. The current study found that the number of steps in the L group were higher during the weeks in the cubicle pen and decreased more prominently in the first week in the straw yard compared to the H group. Potential reasons for this discrepancy are uncertain, but probably related to the different lying space allowances between the two studies: lying space 6.0m^2 versus 12.0+ m^2 for the current study, 3.3 m^2 versus 7.8m^2 for Fustini et al. (2017). No notable treatment effect was observed in the pattern of change in the activity level during the rest of the treatment period.

More frequent lying bouts and a higher proportion of lying were observed during the weeks in the straw yard compared to the weeks in the cubicle pen. MotionIndex also increased in the first week in the straw yard. This may be due to a change in the floor surface from a hard concrete to a deep straw-bedded floor, which enabled cows to lie down and stand up more easily (Tuyttens, 2005). Additionally, cows may cope better with restricted lying space in straw yards compared to cubicle pens, as they can reduce the inter-cow distance in straw yards (Fregonesi and Leaver, 2002; Fustini et al., 2017).

Days after weekly mixing

Lower proportions of time spent lying and a higher MotionIndex on the day of regrouping showed that cows' daily activity levels were significantly higher on the day of regrouping compared with the day after regrouping. It is possible that cows interacted with unfamiliar cows in places other than the feeding area, which contributed to the increased activity. Indeed, Schirmann et al. (2011) found increased agonistic social interactions on the day of mixing. von Keyserlingk et al. (2008) also reported a significant decrease in lying bouts and lying time on the day of mixing, especially among cows moved to a new group. In the current study, however, new cows joined the treatment groups just after the peak feeding period, and so the competition at the feed-face was less likely to contribute to any increased activity. Instead, this increase in activity level may be related to a weekly farm routine where all dry cows were walked through the footbath, and then returned or moved to the allocated pen. The current study did not find that greater space allowances reduced the change in lying activity on the day of mixing.

Lameness score and parity

The current study showed that overall activity levels were lower in lame cows and multiparous cows. It is plausible to argue that lame cows were less active because of pain in the affected limbs, and that the greater activity level of primiparous cows may be due to a higher level of competitive behaviours at the feed-face. An increased activity in nulliparous cows (including less time lying and more lying bouts) compared with parous cows (parity≥1) was also observed in a study on non-lactating Jersey cows (Lobeck-Luchterhand et al., 2015).

3.4.5. Physiological parameters

In addition to measuring the behavioural implications of the treatment, the current study also investigated physiological parameters relating to stress and metabolic function. High stocking density during the dry period did not affect the concentrations of faecal glucocorticoid metabolites (FGCM) at any sampling points during the dry period. This result was similar to Silva et al. (2016), who found no difference in the prepartum serum concentration of cortisol between 80% and 100% stocking densities (see **Table 1.1**, **p47**). A study on lactating cows (Krawczel et al., 2012a) also found that the concentrations of FGCM were not affected by increasing stocking density from 100% to 113%, 131%, and 142% (see **Table 1.1**, **p47**).

In contrast, Fustini et al. (2017) found that overstocking during the prepartum period (0.66m feed-face and 3.3m^2 of lying area per cow) increased plasma cortisol levels at two weeks before calving compared to a control group (1.65m of feed-face and 7.8m^2 of lying area per cow). Similarly, Huzzey et al. (2012) found a tendency for cows to have a higher concentration of FGCM during the overstocked period (0.34m feed-face and 0.5 cubicle per cow) compared to the control period (0.67m feed-face and 1.0 cubicle per cow), suggesting that high stocking density and an associated increase in agonistic social interactions may have induced physiological stress responses. The lying area of the high stocking group in the current study was less restricted compared to the studies by Fustini et al. (2017) and Huzzey et al. (2012), which may have resulted in the different outcomes in stress responses to high stocking density.

The current study found that the concentrations of FGCM were higher during the dry period than at dry-off regardless of the stocking density. This may indicate a part of a biological adaptation to the transition from late gestation to early lactation (NRC, 2001), but the level of increase was most prominent in the first week after dry-off. Cows can be exposed to various changes in management during the dry period such as abrupt cessation of milking and alterations in diet, group composition and housing, all of which can be stressful to dry cows and may have triggered the activation of the HPA axis. Moreover, weekly regrouping was also a source of stress evident in the increased competition at the feed-face, which may contribute to a higher FGCM during the dry period.

The current study found that stocking density had no significant effect on serum NEFA, BHB and plasma glucose concentrations. Similarly, Silva et al. (2014) and Silva et al. (2016) found that understocking cows at 80% did not affect the concentrations of prepartum glucose, prepartum NEFA and postpartum BHB compared to 100% stocking density. However, Huzzey et al. (2012) found elevated plasma NEFA and glucose concentrations during the overstocked period despite an increased DMI, indicating that overstocking resulted in negative energy balance. They also reported that overstocking affected energy metabolism, evident in a greater resistance to insulin after a glucose tolerance test. The discrepancy between studies may again be due to a harsher experimental setting in the study by Huzzey et al. (2012). Moreover, cows in this study experienced both control and overstocked conditions (see above), which made comparisons between the same animals possible. In contrast, the cows in the current study stayed in the same stocking density throughout the experimental period, so that the group comparison may have masked individual variations in the response to overstocking.

Huzzey et al. (2012) found a parity difference in the physiological responses to overstocking. The metabolic status of the primiparous cows was compromised during the overstocked period compared to the control period, while no change in metabolic status was observed in the multiparous cows. It has been reported that higher stocking densities reduced the feeding time of primiparous cows, whilst no significant change in feeding time was observed in multiparous cows (Huzzey et al., 2012; Lobeck-Luchterhand et al., 2015). These results suggest that the compromised metabolic status of overstocked primiparous cows may be due to a shortened feeding time associated with overstocking. In contrast, the current study found no difference between parities in feeding time during the peak feeding period, which corresponded with other results from the current study that found no evidence of parity differences in the physiological responses to overstocking.

3.4.6. Effect on cow health and production

The current study found that overstocking in a dynamic social environment during the dry period neither increased nor decreased the risks for mastitis and metabolic disorders during the postpartum period. This is in agreement with Silva et al. (2013), which found that a dynamic social environment experienced during the prepartum period had no impact on the incidence of retained placenta, metritis, displaced abomasum or mastitis in the first 60 days postpartum in Jersey cows. Silva et al. (2014) also found that understocking of prepartum Jersey cows (80%) did not affect the incidence of peripartum diseases, displacement of the abomasum and mastitis in the first 60 days postpartum compared with cows at 100% stocking density. The current study also confirmed that locomotion score and body condition score were not influenced by prepartum social environment, which was in agreement with previous studies (Fregonesi and Leaver, 2002; Silva et al., 2014; Fustini et al., 2017). The current study also found that high stocking density and a dynamic social environment during the dry period had no significant impact on milk yield and somatic cell count, which was similar to the results from Silva et al. (2013) and Fustini et al. (2017).

3.5. Conclusion

High stocking density during the dry period altered feeding activity and increased competition at the feed-face. At high stocking densities, cows took longer to approach the feed-face and spent less time feeding during three hours after feed delivery. The activity level of cows was not influenced by stocking density alone, but the effects of housing/feed-face type and parity appeared to be more pronounced with an increased stocking density. The activity level of cows was reduced in the cubicle housing when limited lying space was available, and primiparous cows were more active than multiparous cows. However, the current study suggests that these behavioural changes associated with limited feed-face and lying space allowance were not reflected in the physiological responses of the cows, and that high stocking density during the entire dry period had no impact on postpartum health and productivity. It is possible that the lack of identifiable differences in production, health and physiology between the two differing stocking densities studied here was due to large individual animal variation. In both stocking densities, some cows could be more successful during competition at the feed-face and have better access to feed and lying space than other cows. Nevertheless, the current experimental setting was not stressful enough on the cows to result in observable effects on health and production. There was also no evidence that high stocking density enhanced the activation of the HPA axis, as assessed using faecal glucocorticoid metabolites. Investigating the impact of overstocking at an individual animal level would help determine whether a competitive social environment during the dry period potentially affected not only behaviour but also the welfare of prepartum cows, and which animals would be more susceptible to the negative effects of overstocking.

Chapter 4 :

Impact of maternal high stocking density during the dry period on dairy calf health, behaviour, and welfare

4.1. Introduction

Fetal growth and development can be influenced by prenatal environment, such as maternal stress and nutrition (Weinstock, 2008; Gao et al., 2012; Kamal et al., 2014). Studies on humans and laboratory animals have shown that maternal obesity or undernutrition during pregnancy can result in pregnancy loss or health problems in offspring (Wu et al., 2006; O'Reilly and Reynolds, 2013). Maternal stress during pregnancy can affect fetal brain programming, in which the regions of the brain related to memory, emotions (fear and anxiety) and stress response are affected (Charil et al., 2010). Potential consequences of altered brain development include altered stress responsiveness and/or impaired learning and stress-coping abilities in aversive conditions (Braastad, 1998; Weinstock, 2008). Although the majority of the studies have been conducted on laboratory animals, changes in offspring behavioural and/or physiological responses to stress associated with maternal stress during pregnancy have also been reported in farm animals such as pigs (Otten et al., 2015) and sheep (Roussel-Huchette et al., 2008).

Pregnant dairy cows can experience various stressors as a part of management practices throughout their gestation period. Cows in the dry period (the non-lactating interval in the last six to eight weeks before calving), in particular, have the potential to experience increased stress due to physiological, metabolic and hormonal changes approaching parturition and changes in management during the dry period. Some studies in cattle have shown that maternal stress during late pregnancy was associated with smaller calf size and altered immune function in offspring (heat stress: Tao et al., 2012a; under nutrition: Gao et al., 2012). Moreover, repeated transportation during pregnancy altered the offspring's physiological response to stress (Lay et al., 1997a). However, the effect of maternal stress on offspring behaviour has not been well investigated.

Dairy calves on commercial farms are normally artificially reared by humans. This unnatural environment requires calves to adapt to challenges associated with management practices, such as an early separation from dams and restricted milk feeding to enhance early starter intake. It would be advantageous if such calves were equipped with a greater ability to adapt to their environment, with stress-coping abilities that are not impaired by prenatal factors. Therefore, this study aimed to investigate the effect of maternal stressful experiences during the dry period on dairy calf performance during the pre-weaning period.

Calves in the current study were born to cows that experienced either a high or low stocking density at the feed-face and the lying area throughout the dry period (see **Chapter 3**). Cows in the high stocking density group took longer to approach and start feeding when freshly delivered feed was put out, and spent more time standing in the alley during the peak feeding period (three hours after feed delivery). A high stocking density resulted in more competition at the feed-face and a reduction in feeding duration during the peak feeding period compared to the low stocking density group.

Based on these results, the current study hypothesised that increased competition for feed and the resulting disturbances in daily feeding pattern experienced by dry cows in the high stocking density group negatively affect offspring performance under commercially relevant situations. The experiment was designed to investigate the effect of maternal treatment on calf body weight, health and growth in the pre-weaning period, the vigour of calves in early life, adaptability of calves to an automated feeding and group housing system, reactions to handling and weaning, and behavioural and physiological responses to disbudding.

4.2. Materials and methods

Calves from cows used in the social stress experiment (see **Chapter 3**) were born between 15th January and 23rd August 2015 at the Crichton Royal Farm SRUC in the Dairy Research and Innovation Centre (Dumfries, UK). All of the animals were managed according to UK regulations on animal care and ethics, and the experimental procedures were approved by the SRUC Animal Welfare and Ethical Review Body (Animal Experiment Number: AE 41-2014). Prior to this experiment, pregnant Holstein Friesian dairy cows were allocated to either high stocking (H) or low stocking (L) treatment groups at dry-off (see **Chapter 3**).

4.2.1. Animals, housing and feeding

Forty-five calves were followed from birth to weaning (H group: n=24, L group: n=21). Two calves (one stillbirth, one flexural limb deformity) and a pair of twins were not included in the experiment. H group comprised 11 beef cross calves (bull=5, heifer=6) and 13 dairy calves (bull=6, heifer=7), and L group comprised 10 beef cross calves (bull=6, heifer=4) and 11 dairy calves (bull=5, heifer=6). All calves were managed in the same way irrespective of maternal treatment. Calves were born in a straw-bedded yard at Acrehead Farm, fed four litres of pooled colostrum via an oesophageal tube within four hours after birth, and stayed with their dams for two to eight hours. Calving was supervised by experienced farm staff, and assistance was provided where necessary by either farm staff or a veterinarian. Calves were then weighed (BW birth) and moved to a straw-bedded individual hutch (1.0m×1.4m×1.2m) with a straw- bedded front yard (1.2m×1.4m) in the calf barn at Crichton Royal Farm. Calves were fed three litres of milk twice a day (8:00 and 15:30) from a bucket with a rubber teat. Non-pasteurised whole milk was provided until the end of February 2015, and milk replacer (VITAMILK Omega Gold: 23.0 % Protein, 18.0%, Oil 18.0%, ForFarmers UK Limited, Bury St Edmunds, UK) was fed from the beginning of March 2015 due to a change in calf feeding management on the farm. Water and calf starter pellets (VITA Start: 18.0% Crude Protein, 11.5% Crude Fibre, Crude Fat 4.0%, 1.0% Ca, 0.5% P, 0.3% Na, 0.3% Mg, Vitamin E 60 IU/kg, ForFarmers UK Limited) were provided *ad libitum* from buckets on the front wall of the yard. Day1 (d1) was defined as when calves first received milk other than colostrum in the hutch, and they stayed in the hutch until d7±1. Halofuginone (Halocur®; MSD Animal Health, Wellington, NZ) was given after morning feeding from d1 to d6 to prevent *Cryptosporidium parvum* infection according to the manufacturer's recommendations.

At d7 or d8, calves were weighed (BW introduction) and moved to a straw-bedded group pen with access to an igloo shed at the end of the pen (**Figure 4.1**) adjacent to the hutches. Calves were moved before the morning feeding (between 7:30 and 10:30) and were then trained to feed from an automatic milk feeder (H&L 100, Holm & Laue GmbH & Co. KG, Westerrönfeld, Germany). Milk replacer was fed from the automatic milk feeder attached to the front wall of the group pen, and a maximum of 6.0 L was provided per day. The maximum milk allowance was set at 1.0 L for each feeder visit to distribute meals throughout the day, and no milk was dispensed to the same calf in the following 100 minutes after the calf had consumed 1.0 L. Calf starter pellets and straw were provided *ad libitum* from troughs attached to the pen wall, and water was available from an automatic water dispenser. Group structure was dynamic with calves entering and leaving depending on date of introduction from the hutches and subsequent weaning dates. Group size ranged between two and 15 calves per group, except for the period from 2nd to 18th of March 2015 when two group pens were combined due to a shortage of milk feeders. The double-sized group pen had one milk feeder, two straw and starter troughs and two automatic water dispensers with the maximum group size being 25 calves.

Calves over 28 days old were disbudded with an electric dehorner by a veterinarian. Cornual nerves were anaesthetised 10 minutes prior to disbudding with 3.0 ml of 50mg/ml Procaine Hydrochloride (Adrenacaine, Norbrook® Laboratories (BG) Ltd, Northamptonshire, UK), and no separate analgesics were administered. Weaning was completed at 49 days of age with a gradual reduction in daily milk allowance from 6.0 L to 0.0 L over 10 days. After weaning, calves were weighed (BW wean) and removed from the group.

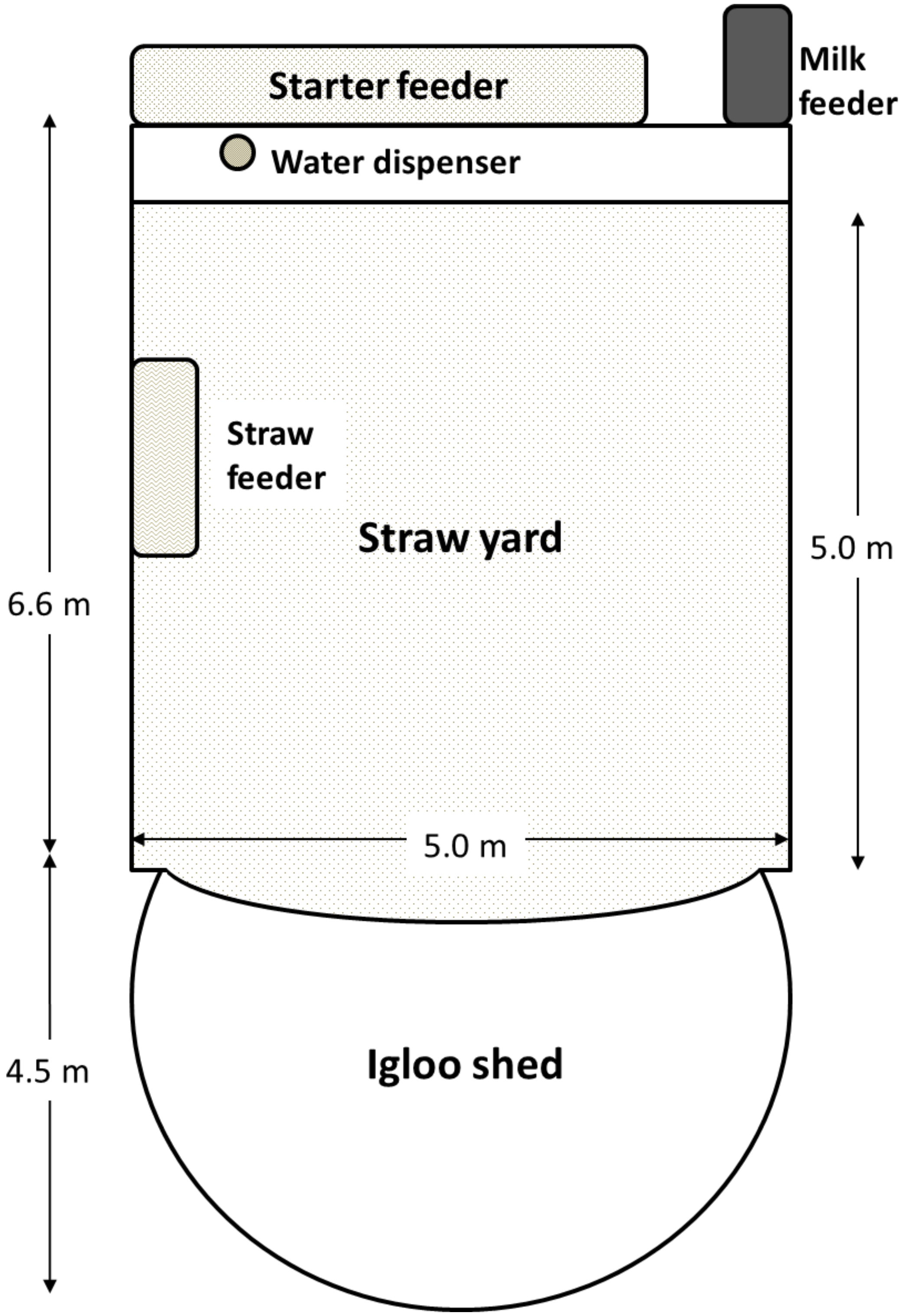

Figure 4.1. Layout of the calf group pen (pen No. 1, 3, 5). Group pen No. 2, 4 and 8 were a mirror image.

4.2.2. Data collection

4.2.2.1. Health recording

The health condition of calves was monitored every day by farm staff, and any incidences of disease and associated treatments were recorded.

4.2.2.2. Calf growth rates

Average daily gains (ADG; g/day) in the hutch, in the group and in the pre-weaning period were calculated as follows:

- ADG hutch (g/day) = (BW introduction – BW birth)/(age at introduction – 1)
- ADG group (g/day) = (BW wean – BW introduction)/days in the group pen
- ADG pre-weaning (g/day) = (BW wean – BW birth)/age at weaning

4.2.2.3. Immunoglobulin level

On d7, blood was collected from the jugular vein into a 10-ml sterile plain tube to measure the immunoglobulin levels of calves. Blood samples were centrifuged at 3000×*g* for 10 min, and sera were stored at -20 ºC until analysis. Concentrations of serum immunoglobulin G (IgG) were measured using a commercial kit (Bovine IgG ELISA kit; Biopanda Reagents, Belfast, UK) according to manufacturer's instructions.

4.2.2.4. Behavioural data

All of the behavioural observations were performed by a single observer after a training period, in which video recordings of ten calves were watched three times until more than 90% agreement of the measures was achieved.

<u>Neonatal behaviour</u>

Waterproof infrared CCTV cameras (1/3" Sony Color CCD, EZ420IR-30, ezCCTV.com Ltd, Herts, UK) were attached to the walls of the calving pen, monitoring the calving process. The cameras were connected to a digital video surveillance system (GeoVision, version8, GeoVision Inc., Taipei, Taiwan) which stored the video footage. The video recordings were watched for each calf for 210 minutes after birth. Birth time was recorded at the full expulsion of the calf, and the time when the calf first performed the following neonatal behaviours were recorded: sternal recumbency, attempt standing, achieve standing, walk, attempt sucking and reach the udder (adopted by Barrier et al., 2012 and modified). Details of the ethogram are described in **Table 4.1**. Latencies of calves to first perform each of the behaviours were calculated. Time of colostrum feeding was also recorded if it occurred during the observation. Calving difficulty (normal birth, calving assisted by farm staff or calving assisted by a veterinarian) was also recorded. Video footage for two calves was missing because they were moved by farm staff to an area where the videos were not monitoring.

Table 4.1. Parameters used to assess neonatal behaviour. Ethogram was adapted from Barrier et al. (2012) and modified.

Sternal recumbency	The calf lies on its sternum with each front leg positioned on each side of its body.
Attempt standing	The calf has its four legs placed under its body, at least one leg fully extended with the ventral part not touching the ground.
Achieve standing	The calf is supported by its four legs, all extended for at least 5 seconds.
Walk	The calf is in a standing position and moves forward with more than two steps.
Attempt suckling	The calf actively attached its muzzle to any parts of dam's body.
Reach the udder	The calf's muzzle is near the udder. Its neck and head are curved up in a sucking position that is maintained for more than 5 seconds.

Activity level

An activity data logger (AX3, Axivity, Newcastle Upon Tyne, UK) was attached to one of the hind legs of each calf from d1 to d9 to measure the activity level. The data logger detects the position of the calf's leg twelve times per second, and an algorithm was used to convert this information to infer its position as lying, standing or intermediate (Haskell, unpublished data). On d9, the data were downloaded and then converted to represent durations (seconds) of lying, standing or intermediate positions using SQL server management studio (Microsoft). The data were then exported to an Excel file where daily lying proportion (LP) was calculated using pivot tables. Activity data for eight calves (H: n=5, L: n=3) were not stored in the data logger for unknown reasons.

Behavioural observations in the group pens

Video cameras (Hi Res Bird Box Camera, 700TVL Sony EFFIO CCD, IR Night Vision, SpyCamera CCTV Ltd., Bristol, UK) were attached to the ceilings and columns of the calf barn and the ceilings of the igloo shed to monitor calf behaviour in the group pen. Video cameras were connected to Geovision (version8, Geovision Inc., Taipei, Taiwan) and video footage from 6 am to 6 pm was stored daily. However, due to a technical problem, the video footage was not stored in the computer between 20th February and 16th March 2015. Calves with missing video footage were not included in subsequent behavioural data analyses.

1) Learning ability and ease of training of calves

On d7 or d8, calves were moved to a group pen with an automatic milk feeder before morning feeding (between 7:30 and 10:30), and were trained to drink from the milk feeder. A training protocol was created to ensure that all calves were taught in the same manner. The trainer presented a hand in front of the calf, letting the calf suck the trainer's fingers or follow the hand. The trainer slowly led the calf into a feeder, or gently pushed the calf from behind if the calf did not follow the hand. If the calf still refused to enter the feeder, one trainer held its body from behind while the other trainer led the calf from the front. Once the calf got into the feeder, the trainer let the calf find a teat on its own, or led the mouth of the calf to the teat. If the calf did not suckle the teat, the trainer held its head or muzzle to make sure the teat was kept in its mouth. Once the calf started drinking, the trainer stayed near the feeder and ensured it consumed the full amount (1.0 L). No training was given to a calf that drank milk on its own before the afternoon feeding time (15:30). The training was continued in the morning (8:00) and in the afternoon (15:30) until the calf drank from the feeder on its own (self-feed). The number of trainings required for calves to self-feed was recorded for each calf (training counts). For three calves, the training count was not recorded because they did not obtain milk during the first training because of a technical problem with the automatic milk feeder.

Video recordings of the first training were watched for all calves (except three calves with the technical issue) to evaluate how easily calves were trained in the first training session. "Ease of training" scores were created (**Table 4.2**) to assess how willingly calves entered the feeder ("willingness to enter the feeder" score) and how easily calves found a teat ("ease of finding a teat" score). The feeder first dispensed 0.4 L of milk (first portion) and then dispensed 0.6 L (second portion) if the calf stayed in the feeder, but there was an interval of up to five seconds between the end of the first portion and dispensing of the second portion. Because of this, some calves stopped drinking just after completing the first portion and subsequently required help to find the teat again. In this case, 0.5 points were added to the "ease of finding a teat" score.

Table 4.2. Ease of training scores.

Willingness to enter the feeder	
0: No help	The calf enters the feeder on its own
1: Easy	The calf enters the feeder by following the trainer's hand (no push)
2: Not Easy	The calf hesitates to enter the feeder so the trainer needs to gently push the calf from behind
3: Difficult	The calf refuses to enter the feeder so the trainer holds its body and pushes into the feeder, or more than one trainer is needed.
Ease of finding a teat	
0.0: No help	The calf finds a teat on its own.
0.5: Additional support	The calf finds a teat on its own, but the calf stops drinking after it consumes the first portion and further help is required to continue to the second portion.
1.0: Easy	The calf finds a teat by following the trainer's hand.
1.5: Easy + additional support	The calf finds a teat by following the trainer's hand, but the calf stops drinking after it consumes the first portion and further help is required to continue to the second portion.
2.0: Not easy	The trainer has to hold the head of the calf or guide it to the teat 2-3 times until the calf starts drinking.
2.5: Not easy + additional support	In addition to 2.0 (Not easy), the calf stops drinking after it consumes the first portion and further help is required to continue to the second portion.
3.0: Difficult	The calf does not keep sucking so the trainer must remain with the calf to repeatedly guide the calf to the teat until it consumes the whole portion. Sometimes two trainers are needed to keep the calf in the feeder.
3.5: Difficult + additional support	In addition to 3.0 (Difficult), the calf stops drinking after it consumes the first portion and further help is required to continue to the second portion.
4: Very difficult	The calf refuses to suck, so the trainer has to squeeze milk for the calf and hold its muzzle to ensure that the calf is drinking, and/or the calf does not consume the whole portion. Sometimes two trainers are needed to keep the calf in the feeder.

2) Reaction to a group environment

Reactions of calves to a novel environment with novel companions were assessed by continuous behavioural observation. The video recordings were watched for each calf. The observation period started when the calf finished its first meal (i.e. when the whole body of the calf was out of the feeder either on its own or having been removed by the trainer), and continued for 30 minutes.

Location in the group pen (milk feeder, straw yard, igloo shed), posture (lying, standing, walking, running, other), behaviour (exploratory, social, self-grooming and other) of calves, and event or state (human intervention, social contact from companions, introduction of a new calf) were recorded by a single person using Observer® XT 12.5 (Noldus Information Technology b.v., Wageningen, The Netherlands). Details of the ethogram are shown in **Table 4.3**.

Latencies of the calf to first perform each of the behaviours and to first enter the igloo sheds were calculated. Time spent in each of the locations and postures, time spent engaging in each of the behaviours (duration), and frequency of the behaviours and the event/state were also calculated. Due to a technical problem, the video footage for ten calves was not stored in the computer, and these calves were not included in the subsequent behavioural analyses.

Table 4.3. Definitions of locations and postures, details of behaviours and event/states used for the observation of calf reactions to a group environment.

Location	
Milk feeder	At least one front foot of the calf is inside the feeder stall.
Straw yard	At least one foot of the calf is in the straw-bedded area.
Igloo	Four legs of the calf cross the front line of the igloo.
Posture	
Standing	The calf stands without leg movement.
Lying	The calf lies down with sternal recumbency or lies on its flank.
Walking	The calf makes a forward movement with more than two steps. Two or three hoofs are touching the ground at any time.
Running	The calf makes a rapid forward movement, including instances of jumping, bucking, galloping and trotting (Jensen, 1999; Krachun et al., 2010).
Other posture	The calf is in a posture not described above.
Behaviour	
Explore (Sniffing/licking/nosing object)	The muzzle of the calf is in contact with or within approximately 5 cm from the wall, floor, chain, straw feeder, water dispenser, starter or any other objects in the pen.
Sniffing/licking/nosing companion(s)-head	The muzzle of the calf is in contact with or within approximately 5 cm from the head of another calf.
Sniffing/licking/nosing companion(s)-belly	The muzzle of the calf is in contact with or within approximately 5 cm from the lower part of another calf's belly.
Sniffing/licking/nosing companion(s)-body	The muzzle of the calf is in contact with or within approximately 5 cm from the body of another calf except under the belly.
Self-grooming	The calf is licking any parts of its own body.
No contact	The muzzle of the calf is not in contact with any objects in the pen, any parts of another calf's body or its own body.
Event/state	
Human inside	There are human(s) inside the pen.
Human outside	There are human(s) outside of the front face of the pen.
Social sniffing/licking	The muzzle of another calf (calves) is (are) in contact or within 5 cm from any parts of the focal calf (lasting 3 seconds or more).
New calf introduction	New calf is introduced into the pen.
Other event/state	Any events not described above occur, or the calf is in a state not described above e.g. urination

3) Behaviour in the first two weeks in the group pen

Activity levels, social and feeding-related behaviours of individual calves were video observed on four different days during the first two weeks in the group pen. Five minute scan sampling was conducted on the first or second day (Gday1), and between three and six days (Gday2) following introduction to the group pen, and another two days randomly selected from the second week in the group pen (Gday3, 4). A pilot video observation revealed that calves' behaviour was affected by farm staff working in the calf barn, which often corresponded to feeding times (7:30-9:00 and 14:30-16:00). Farm staff often stayed in the calf barn after the morning feeding to clean the pens or conduct other routine farm tasks. Therefore, the observation time was selected to be between 13:30 and 16:30, which would better represent the daytime behavioural pattern of calves with minimal human intervention. Each scan sampling recorded calf location, posture, proximity to neighbouring calves and if a calf's muzzle or body was touching other calves (**Table 4.4**). The proportion of time observed in each of the locations and postures, touching a neighbouring calf or human, and being close or not to a neighbouring calf was calculated. The video footage for eleven calves was not stored in the computer due to the aforementioned technical problem.

4) Reaction to weaning

The behavioural reactions to weaning were video monitored for three hours (13:30-16:30) on three occasions: on d40 or d41 (Wday0: before weaning started), on d45 or d46 (Wday1: in the middle of the weaning process) and d49 or d50 (Wday2: when weaning was completed). Location of the calf (straw yard, igloo shed, water dispenser, straw feeder), posture (standing or lying) and human intervention were recorded by five minute scan sampling (**Table 4.4**). The definition of standing was modified to be more suitable for scan sampling; the calf was recorded as "standing" when it was in an upright position including walking and running. Continuous observation was also conducted for three hours to count the number of feeder visits (milk or starter). This is because many of the feeder visits made by the calves around weaning lasted less than five minutes, which could not be recorded by five minute scan sampling. The definition of a starter feeder visit was modified to estimate starter feeding behaviour of calves more accurately. Calves were recorded at the starter feeder when the head of the calf was fully across the line between the straw-bedded area and the starter trough, and remained there for more than five seconds. The end of the starter feeder visit was defined when the head of the calf was away from the starter trough for more than ten seconds. Six calves were not observed because of missing video footage.

Table 4.4. Definitions of locations and postures, details of behaviours and events used for the observation of behaviour in the first two weeks in the group pen and around weaning (except Touch and Proximity).

Location	
Milk feeder	Both front legs of the calf are inside the milk feeder stall.
Starter feeder	The calf's head is within a half body length from the line between the starter feeder and the straw bedded area.
Straw yard	At least one foot of the calf is in the straw bedded area.
Igloo	All four legs of the calf cross the front line of the igloo.
Water dispenser	The muzzle of the calf touches or is within 5 cm from the water dispenser.
Posture	
Standing inactive	The calf is in an upright position with no leg movement.
Lying	The calf lies down in a lateral or a sternal position.
Walking	The calf makes a forward movement with more than two steps. Two or three hoofs are touching the ground at any time.
Running	The calf makes a rapid forward movement, including instances of jumping, bucking, galloping and trotting (Jensen, 1999; Krachun et al., 2010).
Touch	
Social touch actor	The muzzle of the focal calf is in contact with any parts of another calf.
Social touch recipient	The muzzle of other calf (calves) is/are in contact with any body parts of the focal calf.
Mutual touch	The muzzle of the focal calf and another calf are touching each other's bodies or heads.
Non-social touch	Any parts of the calf's body (except muzzle) is in contact with neighbouring calf (calves).
Touching human	The muzzle of the calf is in contact with any parts of human body.
Proximity	
Close	Distance to neighbouring calf (calves) is within one body width.
Not close	Distance to neighbouring calf (calves) is more than one body width.
Human intervention	Human(s) are inside the pen.

4.2.2.6. Reactions to disbudding

Calves were disbudded between 28 and 56 days of age (36.9±7.5SD days of age) at seven occasions with four different veterinarians during the experimental period. All of the disbudding procedures were performed by a veterinarian between 10:00 am and 11:00 am. A calf was put in a crush and its cornual nerves were blocked with local anaesthetic (LA) as previously described. Ten minutes after the administration of LA, the calf was put into the crush again and disbudded with an electric dehorner.

Behavioural observation

1) Behaviour during/after procedure

Calf behaviour during the procedure was recorded using a camcorder. “Willingness to enter a crush” score (adopted by Kilgour et al., 2006) was used to assess reactions of calves to human handling and physical restraint (**Table 4.5**). Calves were scored on two occasions (LA administration and disbudding). Once the whole body of a calf was in the crush, reactions to LA administration (30 seconds) and reactions to the disbudding procedure (1.5 minutes) were observed. The frequencies of head movement, kicking and vocalisation were recorded. The number of times a calf fell down in the crush was also recorded. Details of the behaviour are described in **Table 4.6**.

2) Pain-related behaviour

Frequencies of pain-related behaviour for individual calves were collected by 10 minute live observations approximately 3.5 hours (200±20SD min) and 6 hours (364±13SD min) after the procedure. Behaviours collected were head shaking, ear flicking, head rubbing and self-grooming (**Table 4.6**). Occurrences of rumination, feeding and social behaviour were also recorded.

Change in activity levels

Activity levels (lying proportion: LP) before and after disbudding were monitored using an Activity data logger as previously described, attached to one of the hind legs of a calf 24 hours before and after the procedure. The data were downloaded and then converted for data analyses using SQL server management studio (as previously described). Hourly LP was calculated using pivot tables in Excel.

Physiological data

Salivary samples were collected from all calves using cotton swabs 24 hours before the procedure (baseline), and 30 min, 4 hours and 8 hours after the disbudding. Cotton swabs were centrifuged at 3000×*g* for 15 minutes and extracted saliva was stored at –20 ^{o}C until analysis. Concentrations of salivary cortisol were measured using a commercial kit (Cortisol ELISA (Saliva), ALPCO®, New Hampshire, USA) as per manufacturer’s recommendations.

Table 4.5. Description of the “willingness to enter a crush” score.

Willingness to enter a crush	
0: No help	The calf enters the crush on its own.
1: Easy	The calf is led to the entrance of the crush by the handler, and enters the crush with one or two push(es) by the handler.
2: Not Easy	The calf is led to the entrance of the crush by the handler, but hesitates to enter the crush. The handler needs to push hard, or two people are needed to push the calf into the crush.
3: Difficult	The calf refuses to approach the crush and attempts to escape from the handler. The handler holds the calf body to put it into the crush.
4: Very difficult	The calf refuses to approach the crush and escapes from the handler or jumps out of the crush. The handler(s) holds the calf body and puts it into the crush, but the calf falls down in the crush.

Table 4.6. Details of behaviours used for the observation of reactions to disbudding and pain-related behaviour.

Reactions to local anaesthetic administration and disbudding (video observation)	
Head movement	Distinct movement or twitch of the head despite manual restraint, with/without lifting one or more legs (Graf and Senn, 1999)
Kicking	Quickly lifting a hind hoof up and down, often making a noise when the hoof touched the wall or the floor of the crush
Fall in the crush	Bending both hind legs and sometimes one or both fore leg(s) resulting in the calf not being in an upright position
Vocalisation	The calf vocalising with its mouth open
Pain-related behaviour (live observation)	
Head shaking	Shaking or turning the head without any discernible reason for doing so (Graf and Senn, 1999)
Ear Flicking	Twitching of both ears when no flies present (Faulkner and Weary, 2000)
Head Rubbing	Pushing or rubbing the head with a hind leg or against parts of the pen (Faulkner and Weary, 2000; Heinrich et al., 2010)
Self-grooming	Licking parts of its own body

4.2.3. Data processing and statistical analysis

All of the statistical analyses were performed using Genstat® 16th Edition (VSN International Ltd, Hemel Hampstead, UK) and figures generated using Excel 2013. Test statistics, P-values, means or predicted means, and standard errors of means (SEM) are reported for the data with a normal distribution. Normality of the residuals was checked graphically, and a logit transformation was used where necessary. Back-transformations of predicted means and corresponding 95% confidence intervals [95% CIs] are reported for transformed data. Degrees of freedom (*df*) are reported where it was different from one. Individual statistical models were built for each of the data analyses to examine the effect of treatment, after being adjusted for other variables of interest. Other variables of interest were first tested independently as univariate, and included in the final model if they had a P-value less than 0.25. Characteristics of calves (gender, breed, and maternal parity), disease incidence in the hutch, in the group or in the pre-weaning period and season (winter: January-March, spring: April-June, summer: July-August) were tested in all of the analyses. In addition, other variables were considered for inclusion in order to analyse specific outcome measurements. These variables will be described in the following section. Interactions between treatment and other variables of interest were fitted for all of the analyses by backward stepwise selection, except when using regression models. A post-hoc analysis (Fisher's unprotected least significant difference test) was conducted when there were significant interactions or significant differences between more than two categories (e.g. season) to investigate the direction of the effect.

4.2.3.1. Analysis of descriptive data for calves and neonatal behaviour

General linear models (GLM) were used to analyse BW birth, BW introduction, BW wean, ADG hutch, ADG group, ADG pre-weaning and IgG level at d7. Type of milk fed (whole milk or milk replacer) was tested for the analyses of ADG hutch and group, but not included in the final model (P>0.25). The final models for BW wean, ADG group and ADG pre-weaning included "training count" as a covariate, which was tested independently and had a P-value less than 0.25. The model for IgG included the concentration of colostrum given as covariate, which was also tested independently (P<0.25). An effect of type of milk (whole milk or milk replacer) was tested for ADG in the hutch and IgG level at d7, but it was not significant and was not included in the final model.

The latencies of neonatal behaviour were analysed using proportional hazards (Cox) regression. Proportional hazard regression is a method to investigate the effect of variables on the time at which a specific event occurs. The likelihood of the behaviour occurring was indicated as a hazard ratio (HR). Only two cows received veterinary assistance at calving, and so they were combined with nine cows that were assisted by farm staff. Calving difficulty (normal birth, assisted birth) and colostrum feeding (if colostrum was fed before the behaviour occurred) were included in the final model if the univariate analysis showed $P<0.25$.

4.2.3.2. Lying proportion in the hutch and the group

The activity levels (LP) in the hutch and for the first 48 hours in the group were analysed by linear mixed model using residual maximum likelihood procedure (REML). The data for d1 were often incomplete (less than 24 hours) and therefore were not included in the analysis. The model for LP in the hutch (d2-d6) included treatment, "day in the hutch" and disease incidence in the hutch as fixed effects, and calf as a random effect. Type of milk was tested for LP in the hutch, and not included in the final model as it was not significant. LP in the group pen in the first 48 hours was calculated for each calf, according to the time of introduction to the group pen. Group size (the number of calves in the group pen) and age at introduction to the group pen (d7 or d8) were considered for inclusion in the final model and tested as univariates. The final model for LP in the group pen included an interaction between treatment and "day in the group pen" (group d1: 0-24 hours, group d2: 24-48 hours), disease incidence in the hutch and season as fixed effects. Random effects included calf and treatment nested within pen location (pen/treatment).

4.2.3.3. Training count and ease of training scores

Only three calves received additional support to find a teat, and so the "ease of finding a teat" score was rounded down, which resulted in a five-point scale (0-4). The training count was analysed using REML, and "ease of training" scores were analysed using ordinal logistic regression. In addition to the variables of interest mentioned above, "age at introduction to the group" was tested as a univariate. Group size, which was treated as a covariate, was tested independently in the analysis of the training count. Random effects for the training count were pen/treatment. The final models for the "willingness to enter the feeder" and the "ease of finding a teat" scores were adjusted for the pen location.

4.2.3.4. Reactions to a group environment

The events of "self-grooming", "no social contact" and "new calf introduction" were not included in the analyses because there were very few observations. Sniffing/licking/nosing companion(s) (head, belly, body expect belly) were combined as "social contact initiated", because of very few observations of sniffing/licking belly and other parts of the body. Latencies for each calf to first perform walk, run, explore, enter the igloo shed, lie down, initiate social contact and receive social contact from a pen mate(s) (i.e. social sniffing/licking/nosing) were calculated. Time spent inside the igloo shed and in the straw yard, time spent standing inactive, and time spent engaging in the following behaviours (walk, run, explore and social contact) were also calculated. Frequencies of walking, running, exploring both/either in the igloo shed and/or the straw yard, and social contact (initiated or received) were also calculated.

Latencies for each of the behaviours in the first 30 minutes in the group pen were analysed using proportional hazards (Cox) regression, with the final model being adjusted for the pen location. Durations and frequencies of each of the behaviours in the first 30 minutes in the group pen were analysed using REML including treatment, with the other variables of interest ($P<0.25$ in the univariate analysis) as fixed effects, and pen/treatment as random effects. The duration of "social contact" was analysed following a logit transformation. Additional variables of interest in the analyses of the reactions to a group environment included age of introduction to the group pen, group size (covariate) and whether there were humans inside or outside of the pen during the observation period (yes, no). Two calves stayed in the hutch longer than d7±1 because of their health condition; hence these calves were not included in the analyses.

4.2.3.5. Behaviour in the group pen (in the first two weeks and around weaning)

The data from the five minute scan sampling in the first two weeks in the group pen were first calculated as proportions of the total number of scans: the number of scans for the locations, postures, touching and proximity were divided by the total number of scans (i.e. 37 in total). Proportions that each calf was observed lying, standing inactive and being close to neighbouring calves in the first two weeks were analysed using REML. Proportions of the locations, running and walking, and touching showed skewed distributions and transformation of the raw data did not fulfil the assumption of normality. Therefore, a Generalised linear mixed model (GLMM) was used with a binomial distribution (binomial total=37) and a logit link function. GLMM analyses were unsuitable for the count of running, walking, non-social touching, mutual touching, touching human and water dispenser due to a low number of observations, and these data were removed from the subsequent analyses.

The data from the scan sampling around weaning were processed in the same way as the data for the first two weeks. The count of milk and starter feeder visits from the continuous observation and the proportions of scans that a calf was observed lying were analysed using REML, and the number of times calves were recorded in the igloo and in the straw yard were analysed using GLMM with a binomial distribution (binomial total=37) and a logit link function. The number of times calves were recorded at the water dispenser and the straw feeder were categorised as binary (whether or not calves were observed at the water dispenser and the straw feeder) and analysed using GLMM with a binomial distribution (binomial total=2) and a logit link function.

Observation day (Gday1-4 or Wday0-2) was always included in the final models for both the data in the first two weeks in the group pen and the data around weaning. Additional variables of interest in these analyses included group size (covariate) and presence of human inside (yes or no). The main purpose of the analysis for the behaviour around weaning was to assess the changes in behaviours during the weaning process. Therefore, interactions between observation day and factors/covariates of interest were independently tested, and the final model included the interaction between treatment and observation day, and significant interactions ($P<0.05$ after being adjusted for other variables) as fixed effects. Calf and pen/treatment were fitted as random effects.

4.2.3.6. Reactions to disbudding

Behavioural observation

The willingness to enter the handling crush (for LA and for disbudding) was analysed using ordinal logistic regression. Reactions to LA administration and the disbudding procedure were analysed using REML. The behaviour “fall in the crush” and “vocalisation” were not included in the analyses due to very few observations (n for fall: LA=2, disbudding=4, vocalisation: LA=1, disbudding=2). The frequency of head moving and kicking was summed and analysed together. Previous disease record in the group (treated: yes or no) was considered for inclusion as a fixed effect if a univariate test showed $P<0.25$. Veterinarian ID was included as a random effect. Pain-related behaviours (head shaking, ear flicking and head rubbing) were summed for each of the observations (3.5h and 6h post-disbudding) and analysed using GLMM with a Poisson distribution using a logarithm link function. Veterinarian ID and pen/treatment were included as random effects. The percentage of calves engaged in self-grooming and rumination was analysed using a Chi-square test (3.5h post-disbudding) or Fisher’s exact test (rumination for 6h post-disbudding).

Change in activity levels

Disbudding was performed between 10:00 am and 11:00 am, and changes in the lying proportion over 24 hours after disbudding were compared to the lying proportion on the day before disbudding. The post-disbudding period was divided into the following six periods (0-3h, 4-7h, 8-11h, 12-15h, 16-19h and 20-23h following disbudding), and differences in the lying proportion between pre- and post-disbudding for each of the time periods were calculated. REML was used to analyse the degree of changes in the lying proportion over time. Interactions between time period and factors of interest were first tested independently, and significant interactions ($P<0.05$ after being adjusted for other variables) were left in the final model. Calf and pen/treatment were included as random effects. Veterinarian ID was removed from the random effect as the model did not run when including this factor.

Salivary cortisol levels

The concentrations of salivary cortisol 24h pre-disbudding (baseline), and 0.5h, 4h and 8h post-disbudding were analysed using REML following a logit transformation. The main interest of this analysis was to assess any potential maternal treatment effect on changes in the salivary cortisol level before and after disbudding. Therefore, interactions between sampling timing and factors of interest were first tested independently, and significant interactions ($P<0.05$ after being adjusted for other variables) were left in the final model. Calf, veterinarian ID and pen/treatment were included as random effects.

4.3. Results

4.3.1. BW, ADG and IgG levels

Final models for the analyses of descriptive data are shown in **Table 4.7**.

Table 4.7. Final models used for the analyses of BW, ADG, and IgG.

	Final model
BW birth	*treatment + gender +breed + season*
BW introduction	*treatment + gender +breed + season*
BW wean	*treatment + breed +disease (pre-weaning) + training +season*
ADG hutch	*treatment + parity*
ADG group	*treatment + gender +breed + disease (group) + training count + season*
ADG pre-weaning	*treatment + gender +breed + disease (pre-weaning) + training count + season*
IgG	*treatment + breed +disease (hutch) + colostrum*

There was no significant effect of maternal treatment on BW birth, BW introduction and BW wean. ADG hutch, ADG group and ADG pre-weaning were also not significantly affected by treatment (**Table 4.8**). A gender effect was found in BW birth, with bull calves being significantly heavier than heifer calves (bulls: 48.1±1.1, heifers: 44.2±1.0 kg, W=6.1, P=0.018). There was no significant effect of breed on BW birth (beef-cross: 48.4±1.5, dairy: 43.8±1.4 kg, W=3.3, P=0.075). BW introduction was not significantly affected by gender (bull: 47.1±1.1, heifer: 44.3±0.9 kg, *W*=3.3, P=0.075) or breed (beef-cross: 47.3±1.6, dairy: 44.0±1.4 kg, *W*=1.5, P=0.225). Moreover, no significant effect of breed was found in BW wean (beef-cross: 72.3±2.7, dairy: 67.1±2.3 kg, *W*=1.3, P=0.255). There were no significant effects of maternal parity (*W*=1.4, P=0.237) on the ADG in the hutch. There were also no effects of gender or breed in the ADGs in the group (gender: *W*<0.1, P=0.761; breed: *W*=1.0, P=0.332) and in the pre-weaning period (gender: *W*=0.9, P=0.339; breed, *W*=1.1, P=0.293). Season did not affect BW and ADG.

Table 4.8. Body weights (kg) at birth, at introduction to the group and weaning, and average daily gains (g/day) in the hutch, the group and the pre-weaning period. The figures indicate means±SEM.

		High stocking	**Low stocking**	W statistics	P-Value
Body weight (kg)	birth	45.4±1.0	46.5±1.1	0.6	0.439
	introduction	45.1±0.9	46.1±1.0	0.5	0.492
	weaning	69.0±1.5	69.8±1.7	0.1	0.759
ADG (g/day)	hutch	-25.6±57.1	-5.3±62.6	0.1	0.812
	group	578.7±27.1	587.1±32.0	<0.1	0.844
	pre-weaning	493.2±25.8	489.0±30.3	<0.1	0.915

Respiratory disease and diarrhoea were the main diseases that pre-weaned calves were treated for, and the number of calves treated for illness during the pre-weaning period was not significantly different between the maternal treatment groups (Chi-square value<0.1, P=0.841, **Table 4.9**).

However, there was a significant effect of disease incidence on BW and ADGs. Calves that were treated for illness in the pre-weaning period had a lower body weight at weaning compared to calves that required no treatment for clinical illness (treated: 66.1±1.5kg, not treated: 73.8±1.8 kg, *W*=9.5, P=0.004). Calves that were treated in the group pen grew slower than calves that were not treated in the group pen (treated: 533.0±31.2 g/day, not treated: 631.5±31.2 g/day, *W*=4.4, P=0.045), but disease incidence in the pre-weaning period did not have a significant effect on pre-weaning growth (treated: 458.1±26.7 g/day, not treated: 537.2±31.8 g/day, *W*=3.3, P=0.079).

The serum IgG level at d7 was not significantly different between maternal treatments (High: 7.9±0.8 mg/ml, Low: 8.1±0.9 mg/ml, *W*<0.1, P=0.911) and between breeds (beef-cross:

9.1±0.9 mg/ml, dairy: 7.1±0.8 mg/ml, W=2.9, P=0.100). No significant relationship was found between the colostrum quality given and the serum IgG level of calves (W=2.4, P=0.131). There was also no significant difference in the serum IgG levels in the first week of life between the disease incidence in the hutch (treated: 5.0±1.7 mg/ml, not treated: 8.5±0.6 mg/ml, W=3.3, P=0.081).

Table 4.9. Disease incidence in the hutch, in the group and in the pre-weaning period. Figures indicate the number of calves in each group that were treated or not treated for either respiratory disease or diarrhoea.

		High stocking		**Low stocking**	
		Respiratory	Diarrhoea	Respiratory	Diarrhoea
Hutch	not treated	23	22	20	20
	treated	1*	2	1*	1
Group	not treated	16	15	14	15
	treated	7 (5)	9 (5)	7 (2)	6 (2)

		High stocking	**Low stocking**	Chi-square	P-Value
Pre-weaning	not treated	11	9	0.04	0.841
	treated	13	12		

*One calf from each group was treated both in the hutch and in the group. The figures in brackets indicate the number of calves in each group that were treated for both respiratory disease and diarrhoea.

4.3.2. Neonatal behaviour

Final models for neonatal behaviour data are shown in **Table 4.10**.

Table 4.10. Final models used to analyse neonatal behaviour using Cox regression.

	Final model
sternal recumbency	*treatment + breed + parity + calving difficulty*
attempt standing	*treatment + breed + colostrum feeding + season*
achieve standing	*treatment + breed + colostrum feeding + season*
start walking	*treatment + gender + breed + colostrum feeding + season*
attempt suckling	*treatment + calving difficulty+ colostrum feeding*
reach the udder	*treatment + calving difficulty + colostrum feeding*

Hazard ratios (HRs) obtained from a Cox regression model indicate the likelihood of each of the behaviours occurring within a given time (210 minutes), as opposed to a reference level. There was no difference between maternal treatment groups in the likelihood of calves performing any of the neonatal behaviours (**Table 4.11**). There was a significant effect of breed on the likelihood of calves being in a sternal position, where dairy calves took longer to be in this position compared to beef-cross calves (reference: beef-cross, HR=0.4 [0.2, 0.8], P=0.010). The effect of breed was not observed in the latency to attempt standing (HR=0.8 [0.3, 2.6], P=0.756), achieve standing (HR=1.4 [0.5, 4.4], P=0.568) or start walking (HR=1.2 [0.4, 3.6], P=0.806). There was no significant difference in the likelihood of calves being in a sternal position between calves born to multiparous (parity>2) cows and calves born to primiparous (parity=2) cows (reference: multiparous, HR=0.5 [0.3, 1.1], P=0.066).

All calves were in sternal recumbency when colostrum was fed. Colostrum feeding delayed calves to attempt standing (HR=0.3 [0.1, 0.9], P=0.015), to start walking (HR=0.4 [0.1, 1.0], P=0.029), to attempt suckling (HR=0.1 [<0.1, 0.4], P<0.001), and to reach the udder (HR=0.3 [0.1, 0.8], P=0.007), but did not affect the latency to achieve standing (HR=0.5 [0.2, 1.6], P=0.212). Assisted calving significantly reduced the likelihood of neonatal calves to be in the sternal position (HR=0.3 [0.1, 0.8], P=0.007), but did not affect the likelihood of the other neonatal behaviours occurring. There was no effect of season on the latencies of these neonatal behaviours.

Table 4.11. Median latencies (IQR) of calves performing neonatal behaviours after birth, and hazard ratios [95% CIs] of calves from low stocking maternal treatment in performing the neonatal behaviours within 210 minutes after birth.

	Median (IQR) minutes N calves performed/ N calves observed		**Hazard ratio [95% CIs]**	
	High stocking	**Low stocking**	**Reference=H group**	P-Value
Sternal recumbency	4 (1-7) 23/23	3 (1-5) 21/21	1.1 [0.6, 2.0]	0.881
Attempt standing	42 (31-75)† 21/22	27 (16-46)† 16/19	1.3 [0.6, 2.5]	0.513
Achieve standing	55 (35-118)† 20/22	49 (35-129)† 16/19	0.8 [0.4, 1.6]	0.550
Start walking	64 (44-131)† 20/22	57 (40-153)† 16/19	0.9 [0.4, 1.7]	0.640
Attempt suckling	119 (50-208)† 16/21	78 (41-210)† 13/18	1.4 [0.7, 2.9]	0.413
Reach the udder	160 (65-210)† 13/21	159 (55-210)† 9/18	0.9 [0.4, 2.0]	0.748

†Values for calves that did not perform the behaviour were set at 210.

4.3.3. Activity levels

Final models for LP hutch and LP group are shown in **Table 4.12**.

Table 4.12. Final models used to analyse LP hutch and LP group.

	Final model
LP hutch	*treatment + day (hutch) + disease (hutch)*
LP group	*treatment + treatment.day (group) + day (group) + disease (hutch) + season*

Maternal treatment had no significant effect on the LP from d2 to d6 in the hutch (High: 0.85±0.01, Low: 0.86±0.01, $F_{1,34.4}$=1.0, P=0.338). However, there was a significant effect of day in the hutch ($F_{4,125.4}$=9.3, P<0.001). The LP on d2 was the highest, followed by a significant decrease on d4, d5 and d6 (**Figure 4.2**). Calves that were treated in the hutch lay down for a significantly larger proportion of the day compared to calves that were not treated in the hutch (treated: 0.89±0.02, not treated: 0.82±0.001, $F_{1,33.6}$=10.6, P=0.003). There was no significant difference between treatment groups in the LP in the first 48 hours in the group pen (High: 0.81±0.02, Low: 0.81±0.02, $F_{1,32.6}$<0.1, P=0.859). However, a significant interaction was found between treatment and day in the group pen ($F_{1,33.4}$=6.0, P=0.019). The treatment effect was greater on the first day than on the second day (**Figure 4.3**). L calves increased their lying proportion from the first day to the second day (P=0.017), whilst the LP of H calves did not change from the first to the second day (**Figure 4.3**). The disease incidence in the hutch had a significant effect on the LP in the first 48 hours in the group pen ($F_{1,30.9}$=4.2, P=0.048), where treated calves had a greater lying proportion (0.83±0.03) compared to healthy calves (0.78±0.01). Season did not have a significant effect on LP in the first 48 hours in the group pen, but LP in the winter (0.79±0.02) tended to be lower than in the spring (0.83±0.02, $F_{1,6.7}$=5.2, P=0.059).

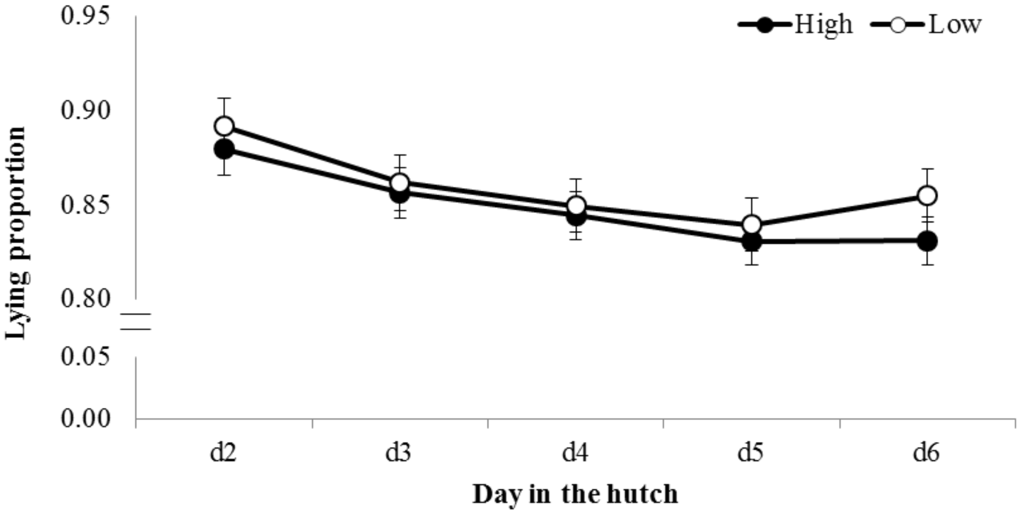

Figure 4.2. Daily lying proportion in the hutch from d2 to d6 (means±SEM).

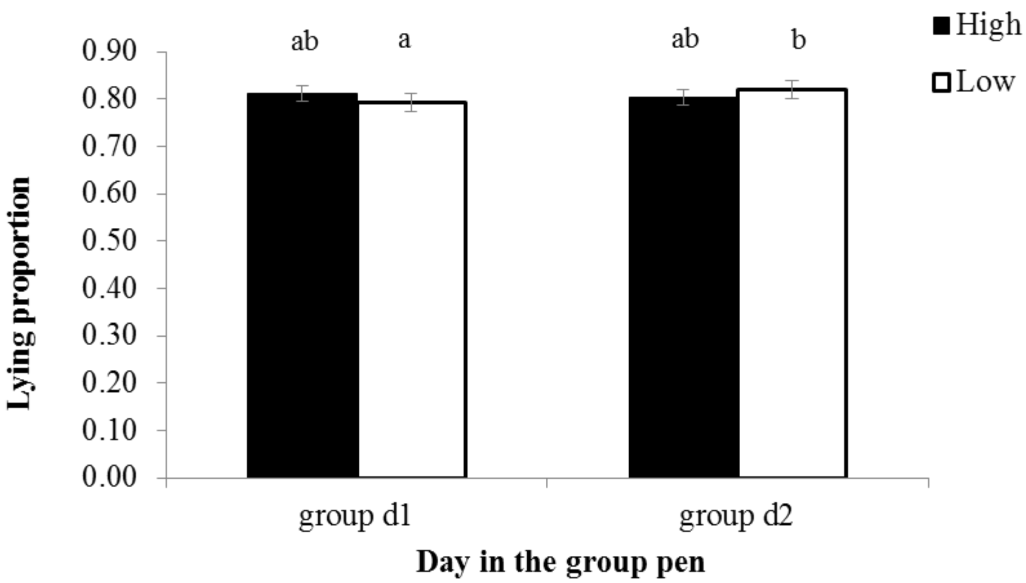

Figure 4.3. Daily lying proportion in the first two days in the group pen (means±SEM). Different letters indicate significant differences (P<0.05).

4.3.4. Learning ability and ease of training of calves

Table 4.13 summarises the models used to analyse the training count and the ease of training scores.

Table 4.13. Models used to analyse training count and the "ease of training" scores.

	Final model
Training count	*treatment + breed + disease (hutch) + season +group size*
Willingness to enter the feeder	*treatment + parity + pen†*
Ease of finding a teat	*treatment + pen†*

†The final model was adjusted for the pen location.

No significant difference was found in the training count between treatment groups (H: 2.2±0.6 times, L: 2.5±0.6 times, $F_{1,32.6}$=0.4, P=0.515). There was no significant effect of breed ($F_{1,10.6}$=0.5, P=0.504), disease incidence in the hutch ($F_{1,31.5}$=1.3, P=0.260), group size ($F_{1,32.3}$<0.1, P=0.835) or season ($F_{2,22.0}$=0.5, P=0.605) on the training count.

However, the training count had a significant negative effect on the average daily weight gain in the group pen, where the average daily gain decreased by 45.7±1.7 g/day for each unit increase in the training count (F=7.0, P=0.013; **Figure 4.4**). High training count was also associated with a lower average daily gain in the pre-weaning period (decrease by 42.1±1.6 g/day per training count, F=3.3, P=0.016) and lower weaning weight (-2.2±0.9 kg per training count, F=5.6, P=0.025).

There was no significant difference between treatment groups in the willingness to enter the feeder (Odds Ratio (OR) for L group=1.3 [0.2, 10.1], t=0.3, P=0.778), or in the ease of finding a teat score (OR for L group=1.9 [0.2, 18.7], t= 0.6, P=0.580). The distribution of calves for the ease of training scores are summarised in **Table 4.14**.

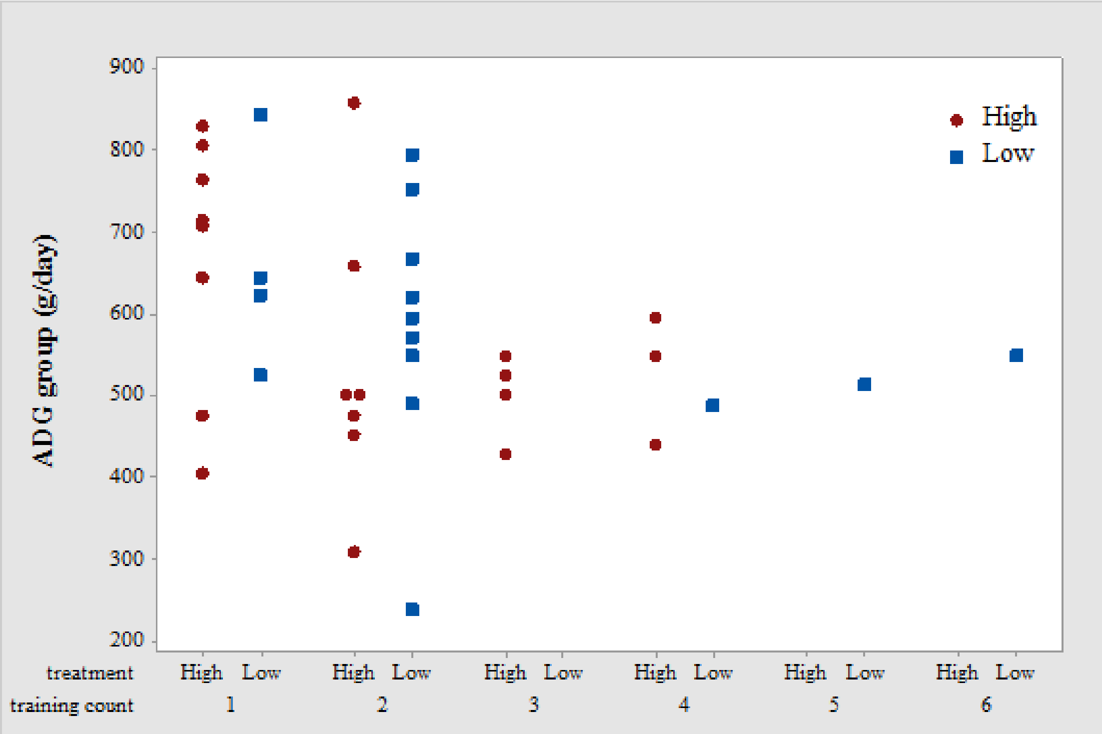

Figure 4.4. Relationship between the training count and average daily gain in the group (g/day). Each symbol indicates an individual animal.

Table 4.14. Distribution of calves for the "willingness to enter the feeder score" and "ease of finding a teat score".

	Willingness to enter the feeder score		**Ease of finding a teat score**	
	High stocking	**Low stocking**	**High stocking**	**Low stocking**
0: No help	0	0	2	0
1: Easy	6	3	13	11
2: Not easy	7	6	4	5
3: Difficult	8	10	0	1
4: Very difficult	NA	NA	2	2

NA: not applicable as no category score 4 for this measure

4.3.5. Reactions to a group environment

The final models used to analyse the reactions to a group environment are summarised in **Table 4.15**.

Table 4.15. Final models used for the analysis of reaction to a group environment.

	Final model
Latency	
walk	*treatment + age + disease (hutch) + season + human inside +pen†*
run	*treatment + parity + age +pen†*
explore	*treatment + pen†*
enter the igloo shed	*treatment + human inside + pen†*
lie down	*treatment + gender + age + disease (hutch) + pen†*
initiate social contact	*treatment + disease (hutch) + group size + pen†*
receive social contact	*treatment + gender + parity + pen†*
Duration	
stand inactive	*treatment + age + human inside + human outside*
walk	*treatment + treatment.group size + human inside + group size*
run	*treatment + treatment.group size + breed + parity + season + size*
explore	*treatment + gender + disease (hutch) + age*
social interaction	*treatment + disease (hutch) + age*
straw yard	*treatment + parity + disease (hutch)*
igloo shed	*treatment + parity + disease (hutch) + age*
Frequency	
walk	*treatment + gender + disease (hutch) + season + human inside*
run	*treatment + group size +season + human outside*
initiate social contact	*treatment + disease (hutch) +age*
receive social contact	*treatment + parity + age + human inside + human outside*
explore	*treatment + gender + disease (hutch) + season + human inside*
explore (igloo)	*treatment + parity + disease (hutch)*
explore (yard)	*treatment + breed*

†The final model for latency was adjusted for the pen location.

Latency

The hazard ratios (HR) and P-values for both maternal treatment groups (obtained from the multivariable analysis) are summarised in **Table 4.16**. There was no difference between treatment groups in the latencies of calves to perform the behaviours mentioned above.

A significant effect of age was observed in the latency to walk (HR=4.7 [1.5, 14.8], P=0.011), run (HR=4.6 [1.4, 15.4], P=0.017), and lie down (HR=4.4 [1.2, 15.7], P=0.028), with calves introduced at d7 (n=29) taking longer to perform these behaviours compared to calves introduced at d8 (n=6).

Calves that were treated for illness in the hutch (n=3) were quicker to walk (HR=4.9 [1.2, 19.8], P=0.047) and quicker to lie down (HR=16.7 [2.9, 96.9], P=0.004) compared to healthy calves (n=32). However, disease incidence in the hutch did not affect the latency of calves to initiate social contact with companions (HR=0.3 [0.1, 1.3], P=0.064).

If a human(s) was (were) inside the group pen, calves started walking sooner compared to when there was no human inside (HR=3.1 [1.3, 7.6], P=0.010), but human presence did not affect the latency for other behaviours to occur. Gender, maternal parity and group size did not affect the latency of any of the recorded behaviours.

Table 4.16. Median latencies (IQR) calculated from raw data for each of the behaviours and hazard ratios [95% CIs] of L calves in performing each of the behaviours during the first 30 minutes in the group pen.

	Median (seconds) (IQR)		**Hazard ratio [95% CIs]**	
Behaviour	**High stocking n=20**	**Low stocking n=15**	**Reference =H group**	**P-Value**
Walk	6.6 (3.0-13.2)	8.4 (3.6-23.4)	0.56 [0.23, 1.33]	0.186
Run	46.8 (24.6-115.8)	42.0 (33.6-160.2)	0.86 [0.36, 2.04]	0.728
Explore	24.0 (15.0-35.4)	16.2 (7.8-97.8)	2.13 [0.97, 4.66]	0.061
Initiate social contact	16.2 (7.8-97.8)	21.0 (6.0-69.6)	0.69 [0.30, 1.58]	0.381
Receive social contact	68.4 (15.6-195.6)	87.6 (34.8-217.8)	1.24 [0.56, 2.72]	0.598
	N calves performed (%)			
Lie down*	19 (95.0)	13 (86.7)	1.63 [0.55, 4.89]	0.380
Enter the igloo shed*	9 (45.0)	8 (53.3)	1.17 [0.49, 2.77]	0.728

*Censoring occurred for the latencies to lie down and enter the igloo shed, hence medians could not be calculated. Number and percentage of calves performing the behaviours are presented.

Duration

There was no significant effect of treatment on the times spent engaging in any of the behaviours in the first 30 minutes after introduction to the group pen (**Table 4.17**). However, there was a significant interaction between treatment and group size on the duration of walking ($F_{1,30.0}$=7.6, P=0.010) and running ($F_{1,27.0}$=5.8, P=0.023). Compared to H calves, L calves spent less time walking (-0.3±0.1 min) and running (-0.1±0.1 min) for each unit increase in group size. A significant effect of age was found on the duration of standing inactive ($F_{1,27.7}$=15.2, P<0.001) and being in contact with companions ($F_{1,30.2}$=5.2, P=0.030), with calves introduced at d7 spending a longer time standing inactive (10.3±1.0 min) and being in contact with companions (1.4 min [0.9, 2.2]) compared to calves that were introduced at d8 (standing inactive: 5.3±1.5 min, social contact: 0.7 min [0.3, 1.4]). Age at introduction did not have a significant effect on any other behaviours.

Calves that were treated for illness in the hutch spent significantly less time making social contact with companions (0.6 min [0.3, 1.4]) compared to healthy calves (1.5 min [1.1, 2.2], $F_{1,29.2}$=5.1, P=0.031). Disease incidence in the hutch did not significantly affect the other behaviours. There was no effect of maternal parity on the duration of running (Primi: 1.6±0.4, Multi: 1.2±0.3 min, $F_{1,27.0}$=2.3, P=0.140), and the time spent in the straw yard (Primi: 16.0±2.7, Multi: 20.7±2.2 min, $F_{1,29.7}$=3.7, P=0.065) and in the igloo shed (Primi: 12.3±2.9, Multi: 7.8±2.5 min, $F_{1,24.3}$=3.4, P=0.076). No significant effect of gender was also observed on the duration of exploratory behaviour (bulls: 9.2±1.3, heifers: 7.2±1.1 min, $F_{1,12.7}$=3.4, P=0.088).

The total running duration for dairy calves was significantly longer than for beef-cross calves (dairy: 2.0±0.3, beef-cross: 0.8±0.3 min, $F_{1,27.0}$=5.7, P=0.024), and calves spent more time running in winter compared to spring and summer (spring: 1.1±0.4, summer: 0.9±0.6, winter: 2.3±0.3 min, $F_{2,27.0}$=3.7, P=0.039). If humans were observed in the pen during the observation period, calves spent less time standing inactive (6.4±1.4 min) and spent more time walking (6.7±0.4 min), compared to when humans were not observed in the pen (standing: 9.3±1.1 min, $F_{1,27.7}$=6.2, P=0.020; walking: 4.9±0.4 min, $F_{1,30.0}$=9.3, P=0.005). The duration of standing inactive was not affected by human presence outside the pen ($F_{1,26.9}$=0.4, P=0.553).

Frequency

There was no significant difference between treatment groups in the frequency of walking, running, social and exploring behaviours in the 30 minutes after introduction to the group pen (**Table 4.17**). However, the frequency of receiving social contact was almost significant

($F_{1,29.0}$=4.2, P=0.051), where L calves tended to receive social contact more frequently than the H calves. A significant age effect was observed in the frequency of social behaviours. Calves that were moved to the group pen on d7 initiated social contact with companions more frequently (16.8±3.3 times) and also received social contact from companions more frequently (18.4±1.2 times) compared to calves that were moved on d8 (initiated: 7.1±5.3 times, $F_{1,30.3}$=4.5, P=0.042; received: 11.5±2.8 times, $F_{1,29.0}$=5.7, P=0.024). There was no significant effect of group size on the frequency of running (decrease by 0.69±0.37 times per unit increase in group size, $F_{1,27.9}$=3.4, P=0.074).

Calves that were treated for illness in the hutch initiated social contact significantly less often compared to healthy calves (not treated: 18.8±2.6, treated: 5.0±6.3 times, $F_{1,29.3}$=5.2, P=0.031). However, disease incidence in the hutch did not affect the frequency of walking (not-treated: 57.8±5.3, treated: 35.3±12.5 times, $F_{1,28.0}$=3.5, P=0.072) and exploratory behaviours (total: $F_{1,28.0}$=1.3, P=0.263, straw yard: F=2.8, P=0.105). Gender, breed, maternal parity and season did not significantly affect the frequency of any behaviours in the first 30 minutes in the group pen. Human presence outside the pen reduced the frequency of calves receiving social contact from their companions (11.4±1.6 times), compared to when no humans were observed outside the pen (18.5±2.5 times, $F_{1,29.0}$=6.4, P=0.017). Human presence inside or outside of the pen did not significantly affect the frequency of other behaviours.

Table 4.17. Time spent on each of the behaviours, the location (duration) and the frequency of each of the behaviours observed in the first 30 minutes in the group pen. Figures indicate predicted means±SEM or back-transformed means [95%CIs] for the data analysed following a logit transformation.

	High stocking	**Low stocking**	W statistic	P-Value
Duration (minutes)				
Standing inactive	7.7±1.2	8.0±1.2	0.1	0.722
Walking	5.6±0.4	5.9±0.5	0.2	0.686
Running	1.6±0.4	1.4±0.4	0.1	0.729
Exploring	7.5±1.2	8.9±1.2	1.7	0.257
Social interaction	1.1 [0.7, 2.0]	0.8 [0.5, 1.5]	1.9	0.179
Straw yard	17.9±2.3	18.7±2.6	0.1	0.725
Igloo shed	10.3±2.6	9.8±2.8	0.1	0.833
Frequency (count/30min)				
Walking	44.9±7.4	48.1±8.8	0.2	0.638
Running	20.9±3.8	21.4±4.0	<0.1	0.923
Exploring	47.0±8.2	56.9±9.8	1.8	0.194
Exploring (straw yard)	25.1±6.2	35.6±7.1	2.6	0.117
Exploring (igloo shed)	19.5±4.8	17.8±5.1	0.1	0.822
Social contact initiated	12.9±4.0	10.9±4.3	0.3	0.570
Social contact received	12.8±1.7	17.1±2.0	4.2	0.051

4.3.6. Behaviour in the first two weeks in the group pen

The final models used to analyse behaviour in the first two weeks in the group pen are summarised in **Table 4.18**.

Table 4.18. Final models used for the analysis of behaviours in the first two weeks in the group pen.

	Final model
lying	*treatment + observation day + gender + disease (hutch) + human inside + season*
standing inactive	*treatment + observation day + gender + disease (hutch) + human inside + season*
close to neighbour calves	*treatment + observation day + gender + breed + disease (hutch) + group size + season*
milk feeder	*treatment + observation day + group size + season*
starter feeder	*treatment + observation day + human inside*
igloo shed	*treatment + observation day*
straw yard	*treatment + observation day + season*
social touch actor	*treatment + observation day + parity + disease (hutch) + season*
social touch recipient	*treatment + treatment.group size + observation day + gender + breed + season + group size*

There was no significant treatment effect on the proportion of time lying (H: 0.67±0.03, L: 0.74±0.03, $F_{1,5.9}$=4.7, P=0.075) and time standing inactive (H: 0.29±0.02, L: 0.24±0.03, $F_{1,5.5}$=3.1, P=0.132). There was also no significant effect of gender on the proportion of time lying (bull: 0.67±0.03, heifer: 0.74±0.03, $F_{1,9.8}$=4.5, P=0.060) and time standing inactive (bull: 0.30±0.03, heifer: 0.23±0.03, $F_{1,7.9}$=4.7, P=0.061). A significant seasonal effect was found on the proportion of time spent lying ($F_{2,23.9}$=3.6, P=0.045), with the highest lying proportion being observed in summer (0.80±0.06), compared to spring (0.68±0.02, P=0.042) and winter (0.63±0.03, P=0.013). Season did not affect the proportions of time spent standing inactive ($F_{2,21.7}$=2.4, P=0.119).

The presence of humans did not affect the proportion of time spent lying ($F_{1,120.8}$=2.7, P=0.104) and time spent standing inactive ($F_{1,119.7}$=3.3, P=0.073). There was no effect of observation day on the proportion of time spent lying (Gday1: 0.70±0.03, Gday2: 0.73±0.03, Gday3: 0.70±0.03, Gday4: 0.70±0.03, $F_{3,115.9}$=0.3, P=0.829) and standing inactive (Gday1: 0.25±0.03, Gday2: 0.24±0.03, Gday3: 0.29±0.03, Gday4: 0.29±0.03, $F_{3,116.0}$=0.9, P=0.438). There was no effect of disease incidence on the proportions of time spent lying ($F_{1,109.9}$=0.6, P=0.427) or standing inactive ($F_{1,103.2}$=1.2, P=0.270).

No treatment effect was observed on the number of times a calf was observed in the milk feeder ($F_{1,124.0}$<0.1, P=0.928), at the starter feeder ($F_{1,126.0}$<0.1, P=0.877), in the igloo shed ($F_{1,34.8}$=0.6, P=0.460), and in the straw yard ($F_{1,31.8}$=0.4, P=0.534). There was no statistically significant effect of observation day on the presence at any of these locations (milk feeder: $F_{3,124.0}$=1.4, P=0.257; starter feeder: $F_{3,126.0}$<0.1, P=0.988; igloo shed: $F_{3,96.7}$=1.0, P=0.414; straw yard: $F_{3,96.2}$=1.2, P=0.316). Group size, season and human presence also did not affect the probability of calves being observed at any of the locations.

There was a significant effect of treatment on the number of social touching events as an actor ($F_{1,40.5}$=9.7, P=0.003), where H calves were more frequently observed touching other calves compared to L calves (**Figure 4.5**). The number of social touching events as a recipient was not different between treatments ($F_{1,113.1}$=0.3, P=0.575, **Figure 4.5**). However, a significant interaction was found between treatment and group size on the number of social touching events as a recipient ($F_{1,117.9}$=5.6, P=0.020). Compared to H calves, L calves were more likely to receive social touching from companions as group size increased (increase by 0.02±0.03 probability per unit increase). There was no effect of observation day on the number of social touching events as an actor ($F_{3,101.3}$=0.2, P=0.914) or as a recipient ($F_{3,116.0}$=2.2, P=0.533). Parity, disease incidence and season did not affect the number of social touching events as an actor, and gender, breed and season had no impact on the number of social touching events as a recipient.

Treatment did not affect the proportion of time spent close to a neighbouring calf (H: 0.3±0.03, L: 0.30±0.03, $F_{1,117.7}$=1.8, P=0.187). A significant gender effect was found on the proportion of calves being close to another calf ($F_{1,25.0}$=5.8, P=0.024), where bull calves were more likely to be observed as being close to their neighbour calves (0.35±0.03) compared to heifer calves (0.27±0.03). The probability of calves being close to their neighbouring calves increased by 0.007±0.003 for each unit of increase in group size ($F_{1,68.4}$=5.3, P=0.024). The probability of calves being close to their neighbouring calves was not affected by observation day ($F_{3,116.0}$=2.0, P=0.115), breed ($F_{1,39.5}$=0.1, P=0.771), disease incidence ($F_{1,117.6}$=0.7, P=0.413), or season ($F_{2,93.0}$=0.4, P=0.708).

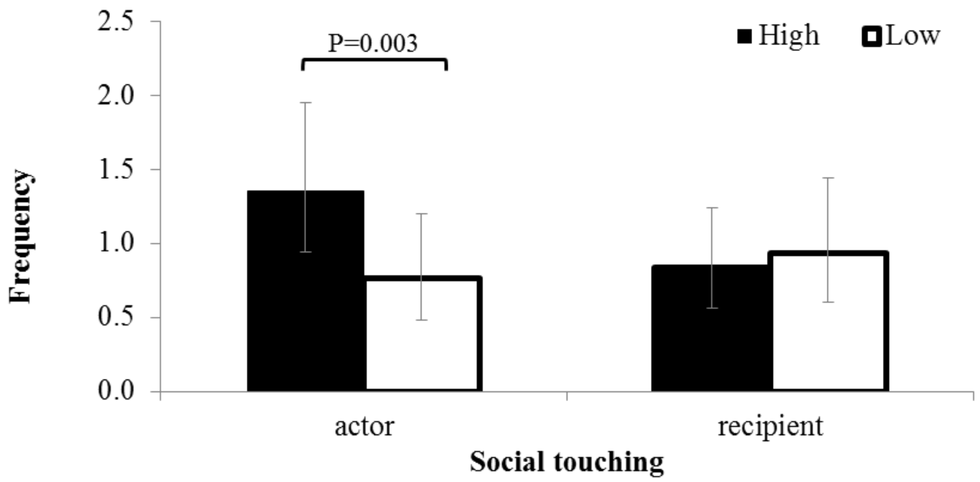

Figure 4.5. The number of social touching events observed as an actor or as a recipient (back- transformed means). Error bars indicate 95% CIs.

4.3.7. Reactions to weaning

Table 4.19 summarises the fixed models used to analyse behaviour around weaning.

Table 4.19. Final models used for the analysis of behaviours around weaning.

	Final model
lying proportion	*treatment + treatment.observation day + observation day*
milk feeder	*treatment + observation day*
starter feeder	*treatment + observation day*
igloo shed	*treatment + treatment.observation day + breed.observation day + breed + observation day*
straw yard	*treatment + treatment.observation day + observation day*
water dispenser	*treatment + observation day*
straw feeder	*treatment + observation day*

There was no difference in the overall lying proportion between the H group (0.57±0.02) and the L group (0.58±0.03, $F_{1,31.4}$=0.1, P=0.831), but a significant interaction was found between treatment and observation day ($F_{2,71.5}$=3.4, P=0.039). The lying proportion for H calves was significantly decreased during the middle of the weaning process (Wday1), compared to the day before weaning started (Wday0) and the day weaning completed (Wday2), but this difference was not observed in the L group (**Figure 4.6**). This resulted in a significant treatment effect on Wday1 (t=2.1, P=0.045), where H calves had a lower lying proportion (0.49±0.04) compared to L calves (0.60±0.04). The number of feeder visits was not affected by treatment (milk feeder: $F_{1,31.6}$=0.1, P=0.733, starter feeder: $F_{1,34.0}$=0.3, P=0.596). However, there was a significant effect of observation day on the number of feeder visits (milk feeder: $F_{2,71.2}$=7.7, P<0.001; starter feeder: $F_{2,71.5}$=6.6, P=0.002). The number of milk and starter feeder visits significantly increased from Wday0 to Wday1 and 2 (**Figure 4.7**).

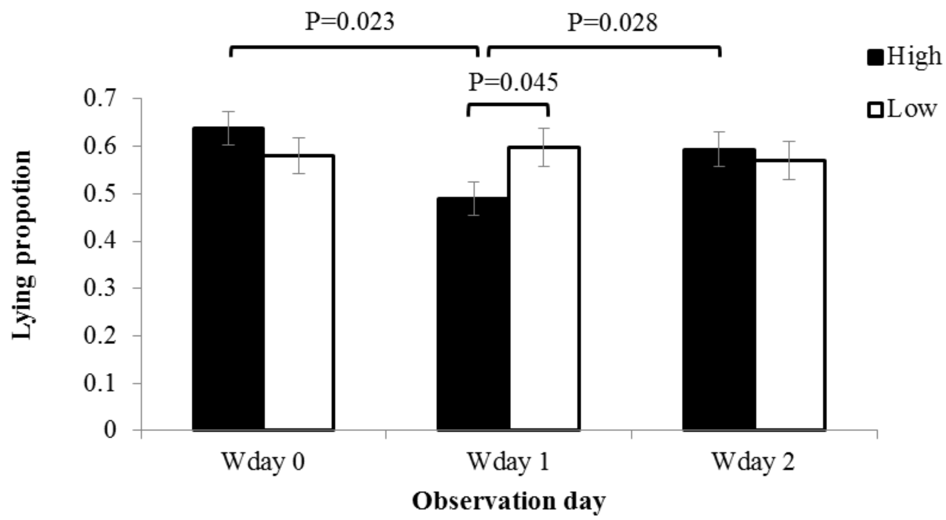

Figure 4.6. Mean lying proportion (±SEM) before weaning started (Wday0), in the middle of weaning (Wday1) and when weaning was completed (Wday3).

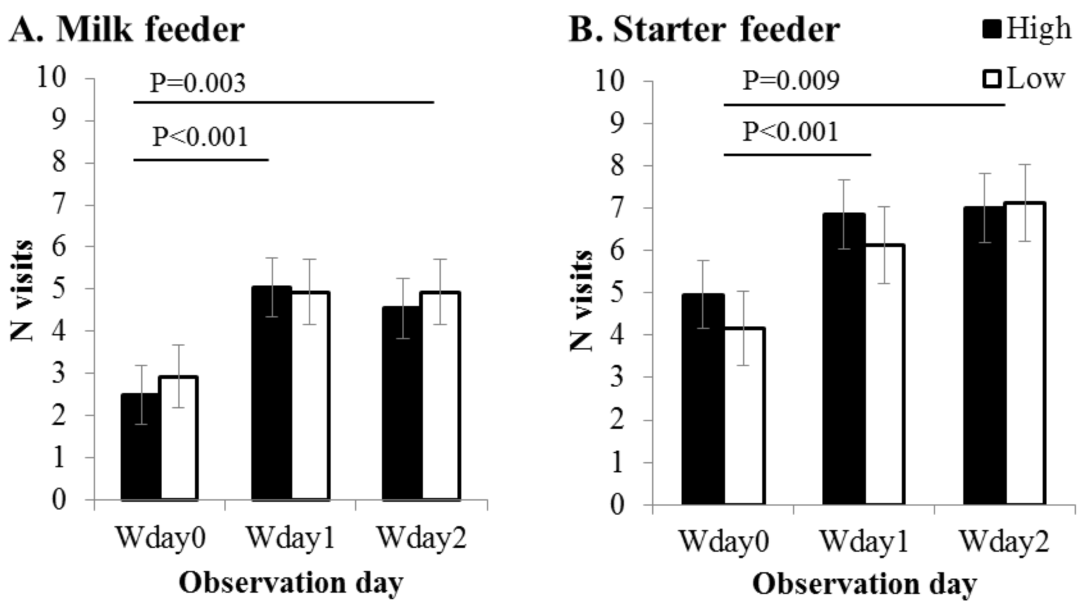

Figure 4.7. The number of milk feeder (A) and starter feeder (B) visits (predicted means±SEM) before weaning started (Wday0), in the middle of weaning (Wday1) and when weaning was completed (Wday3).

There was no treatment effect on the number of times a calf was observed in the straw yard ($F_{1,33.2}$<0.1, P=0.952) and in the igloo shed ($F_{1,31.8}$=0.2, P=0.645). There was a significant effect of observation day in the number of times a calf was observed in the straw yard (Wday0: 14.8 [11.8, 17.9], Wday1: 22.9 [19.6, 25.9], Wday2: 19.0 [15.7, 22.3], $F_{2,71.4}$=6.4, P=0.003), and in the igloo shed (Wday0: 16.5 [12.6, 20.5], Wday1: 5.5 [3.4, 8.7], Wday2: 8.1 [5.3, 11.9], $F_{2,71.0}$=14.0, P<0.001). Moreover, a significant interaction between treatment and observation day was found in the number of times a calf was observed in the straw yard ($F_{2,71.4}$=3.6, P=0.033), and in the igloo shed ($F_{2,71.1}$=3.8, P=0.028). Calves were more frequently observed in the straw yard on Wday1 compared to Wday0 in both H (t=2.6, P=0.015) and L (t=2.7, P=0.012) groups (**Figure 4.8A**), and the opposite pattern was found in the igloo shed (H: t=3.6, P=0.001, L: t=3.4, P=0.002, **Figure 4.8B**).

However, when weaning was completed, the number of times L calves were observed in the igloo shed and in the straw yard returned to the same level as the day before weaning started, whilst H calves were still more likely to be observed in the straw than in the igloo shed. This resulted in a significant difference between the treatments groups on Wday2 in the number of times calves were observed in the igloo shed (t=2.3, P=0.029) and the straw yard (t=2.1, P=0.044).

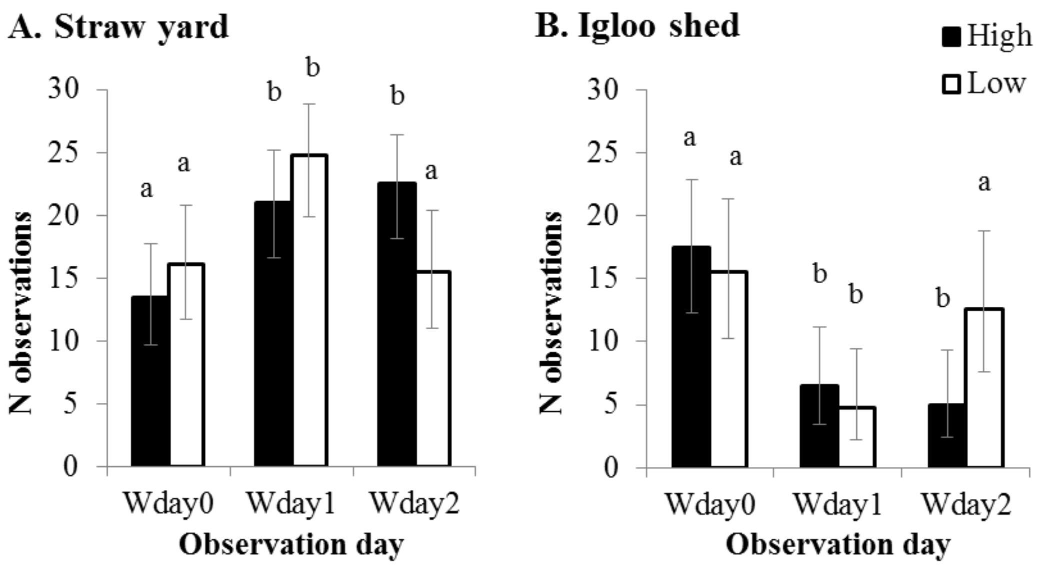

Figure 4.8. The number of times a calf was observed in the straw (A) and in the igloo shed (B) (back-transformed means and 95% CIs) before weaning started (Wday0), in the middle of weaning (Wday1) and when weaning was completed (Wday3). In each figure, different letters indicate significant differences (P<0.05).

There was a significant interaction between breed and observation day on the number of calves observed in the igloo shed ($F_{2,71.1}$=3.8, P=0.045). The number of observations in the igloo shed decreased for both dairy and beef-cross calves from Wday0 (dairy: 16.6 [11.9, 21.6], beef-cross: 16.4 [10.5, 22.7]) to Wday1 (dairy: 3.7 [1.8, 7.3], beef-cross: 8.0 [4.2, 13.9]). On Wday2, the number of observations in the igloo shed for beef-cross calves returned to the same level as Wday0 (13.4 [8.0, 20.0]), whilst dairy calves still had a significantly lower number of observations in the igloo shed compared to Wday0 (4.5 [2.3, 8.4], t=4.4, P=0.001), which resulted in a significant breed difference on Wday2 (t=2.7, P=0.012).

There was no significant effect of treatment on the probability of calves being observed at the water dispenser (H: 0.35 [0.15, 0.61], L: 0.37 [0.16, 0.64], $F_{1,101.0}$<0.1, P=0.855) and the straw feeder (H: 0.41 [0.29, 0.54], L: 0.48 [0.35, 0.62], $F_{1,101.0}$=0.6, P=0.445). There was also no significant effect of observation day on the probability of calves being observed at the water dispenser ($F_{2,101.0}$=2.1, P=0.122) and the straw feeder ($F2_{,101.0}$=0.9, P=0.422), although there was an increase from Wday0 to Wday1 and Wday2 (water dispenser: Wday0: 0.23 [0.09, 0.47], Wday1: 0.46 [0.23, 0.71], Wday2: 0.40 [0.19, 0.66]; straw feeder: Wday0: 0.36 [0.23, 0.52], Wday1: 0.47 [0.31, 0.63], Wday2: 0.49 [0.33, 0.65]).

4.3.8. Reactions to disbudding

Final models used to analyse reactions to disbudding are summarised in **Table 4.20**.

Table 4.20. Final models used for the analysis of reactions to disbudding.

	Final model
Willingness to enter a crush	
Local anaesthesia	*treatment*
Disbudding	*treatment*
Reactions to handling	
Reactions to local anaesthesia	*treatment*
Reactions to disbudding	*treatment + treatment.disease + disease + gender + parity*
Pain-related behaviour	
3.5h post-disbudding	*treatment + treatment.disease + disease + breed*
6h post-disbudding	*treatment + treatment.disease + disease*
Change in activity levels	*treatment + breed + timing + breed.timing*
Change in Salivary cortisol levels	*treatment + breed + timing + breed.timing*

Reactions to handling

There were no treatment differences in the willingness to enter a crush on both occasions (LA: OR for the L group=2.2 [0.7, 7.2], P=0.202; disbudding: OR for the L group=0.8 [0.2, 2.9], P=0.762; **Table 4.21**). There was no difference between treatments in the total number of head movements and kicking behaviours in response to LA administration (H: 3.9±0.5, L: 3.8±0.5 times, $F_{1,39.0}$<0.1, P=0.977) and disbudding (H: 6.2±1.3, L: 8.3±1.3 times, $F_{1,35.0}$=3.3, P=0.076). There was a significant interaction between maternal treatment and previous disease treatment record in the behavioural responses to disbudding ($F_{1,33.6}$=22.5, P<0.001). L calves with no previous disease treatment record in the group pen (non-treated L: n=11) displayed significantly higher behavioural responses to disbudding compared to H calves with no previous disease treatment record (non-treated H: n=9; t=5.0, P<0.001) and L calves with previous disease record in the group pen (treated L: n=8, t=4.5, P<0.001; **Figure 4.9**). Behavioural reactions to disbudding for treated L and treated H (n=13) calves were not significantly different (t=1.8, P=0.077), and previous disease record in the group pen did not significantly affect the behavioural responses of H calves (t=2.0, P=0.060; **Figure 4.9**). Calves born to primiparous cows showed more frequent head movement and kicking during disbudding (9.5±1.1 times) compared to calves born to multiparous cows (7.2±1.1 times, $F_{1,34.0}$=7.4, P=0.010). Gender difference was not observed in the behavioural reactions to disbudding ($F_{1,33.4}$=1.9, P=0.173).

Table 4.21. Distribution of calves for the "willingness to enter a crush" score for local anaesthetic administration and for disbudding.

Willingness to enter a crush score	LA		Disbudding	
	High	Low	High	Low
0: No help	0	0	0	0
1: Easy	3	2	3	1
2: Not Easy	13	9	12	13
3: Difficult	6	4	6	5
4: Very difficult	0	4	1	0

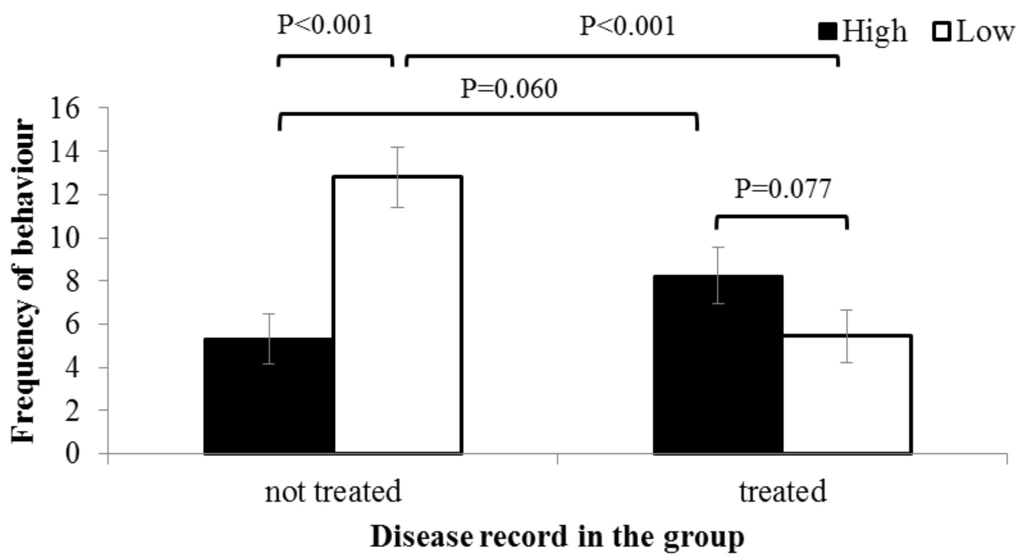

Figure 4.9. Behavioural reactions (mean frequency±SEM) to the disbudding procedure (head movement and kicking) for calves with no previous disease record in the group (not treated) and calves with previous disease record in the group (treated).

Pain-related behaviour

There were no significant differences between treatment groups ($F_{1,37.0}$=2.4, P=0.132), disease incidence category ($F_{1,37.0}$=1.5, P=0.223) or breeds ($F_{1,37.0}$=0.6, P=0.450) on the frequency of pain-related behaviours (head shaking, ear flicking and head rubbing) 3.5h post-disbudding. However, there was an interaction between maternal treatment and disease record in the group pen ($F_{1,37.0}$=5.8, P=0.016). Non-treated L calves displayed significantly more frequent pain-related behaviours compared to non-treated H calves (t=2.7, P=0.036), but the treatment effect was not observed in treated calves (**Figure 4.10**). Although there was no significant difference, previous disease incidence tended to reduce the expression of the pain-related behaviour in L calves (t=2.4, P=0.051) and this was not observed in H calves (**Figure 4.10**). At 6h post-budding, L calves displayed almost two times the frequency of pain-related behaviour (17.7 [6.5, 48.0]) as H calves (9.2 [3.2, 26.4]), but this was not statistically significant ($F_{1,33.1}$=3.4, P=0.076; **Figure 4.10**). There was also no significant interaction between treatment and disease incidence on pain-related behaviour, but a significant effect of previous disease treatment record was observed (non treated: 20.9 [7.7, 56.6]; treated: 7.8 [2.5, 23.8], $F_{1,36.4}$=4.9, P=0.033).

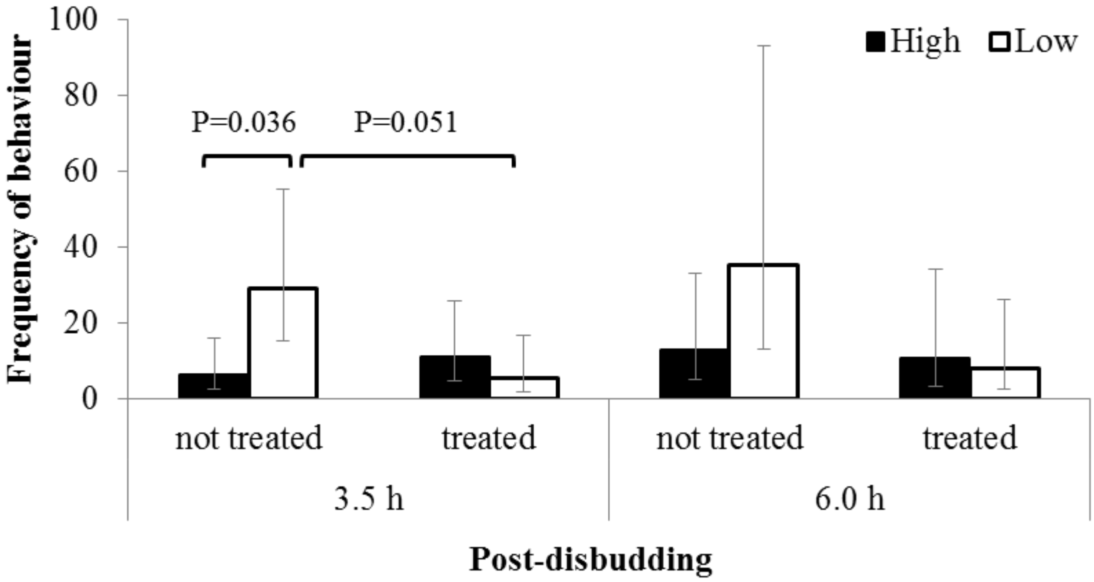

Figure 4.10. Frequency of pain-related behaviours (back-transformed means and 95% CIs) displayed by calves with no previous disease record (not treated) and calves with the previous disease record (treated) during live observations (10 minutes) 3.5h and 6h after disbudding.

The percentage of calves engaged in self-grooming during the 3.5h and 6h post-disbudding observations was not significantly different between treatment groups (3.5h: Pearson Chi-Square=0.173, P=0.687; 6h: Pearson Chi-Square=0.114, P=0.735; **Table 4.22**). The percentage of calves that ruminated during the observations was also not different between groups (3.5h: Pearson Chi-Square=0.602, P=0.435; 6h: P=0.115, *Fisher's exact test*; **Table 4.22**). Social and feeding behaviours were not frequently observed during the 3h (social: n=2, feeding: n=5) and 6.5h (social: n=2, feeding: n=4) post-disbudding observations.

Table 4.22. Percentages (number of observations) of calves that engaged in self-grooming or rumination during the 10 minute observation period after 3.5h and 6h post-disbudding. P-values were obtained from a Chi-square test for self-grooming (3.5h and 6h) and ruminating (3.5h), and from Fisher's exact test for ruminating (6h).

		High (n=22)	Low (n=19)	P-value
Self-grooming	3.5h	59.1 (13)	52.6 (10)	0.687
	6h	31.8 (7)	36.8 (7)	0.735
Ruminating	3.5h	31.8 (7)	21.1 (4)	0.435
	6h	9.10 (2)	31.6 (6)	0.115

Change in the lying proportion between two 24 h periods before and after disbudding

There was no significant effect of treatment on the lying proportion in any of the periods observed ($F_{5,833.3}$=1.7, P=0.144). However, there was a significant difference between the 0-3h period and the rest of the periods ($F_{5,843.3}$=16.6, P<0.001), where the lying proportion immediately after disbudding (0-3h) was lower than the previous day, but it returned to almost the same level as the baseline at 4h post-disbudding (**Figure 4.11A**). A significant interaction between breed and time period was found on the change in the lying proportion ($F_{5,833.5}$=4.2, P<0.001), where beef-cross calves decreased their lying proportion significantly more than dairy calves 0-3h post-disbudding (**Figure 4.11B**). The change in the lying proportion for the rest of the time periods was not different between dairy and beef-cross calves.

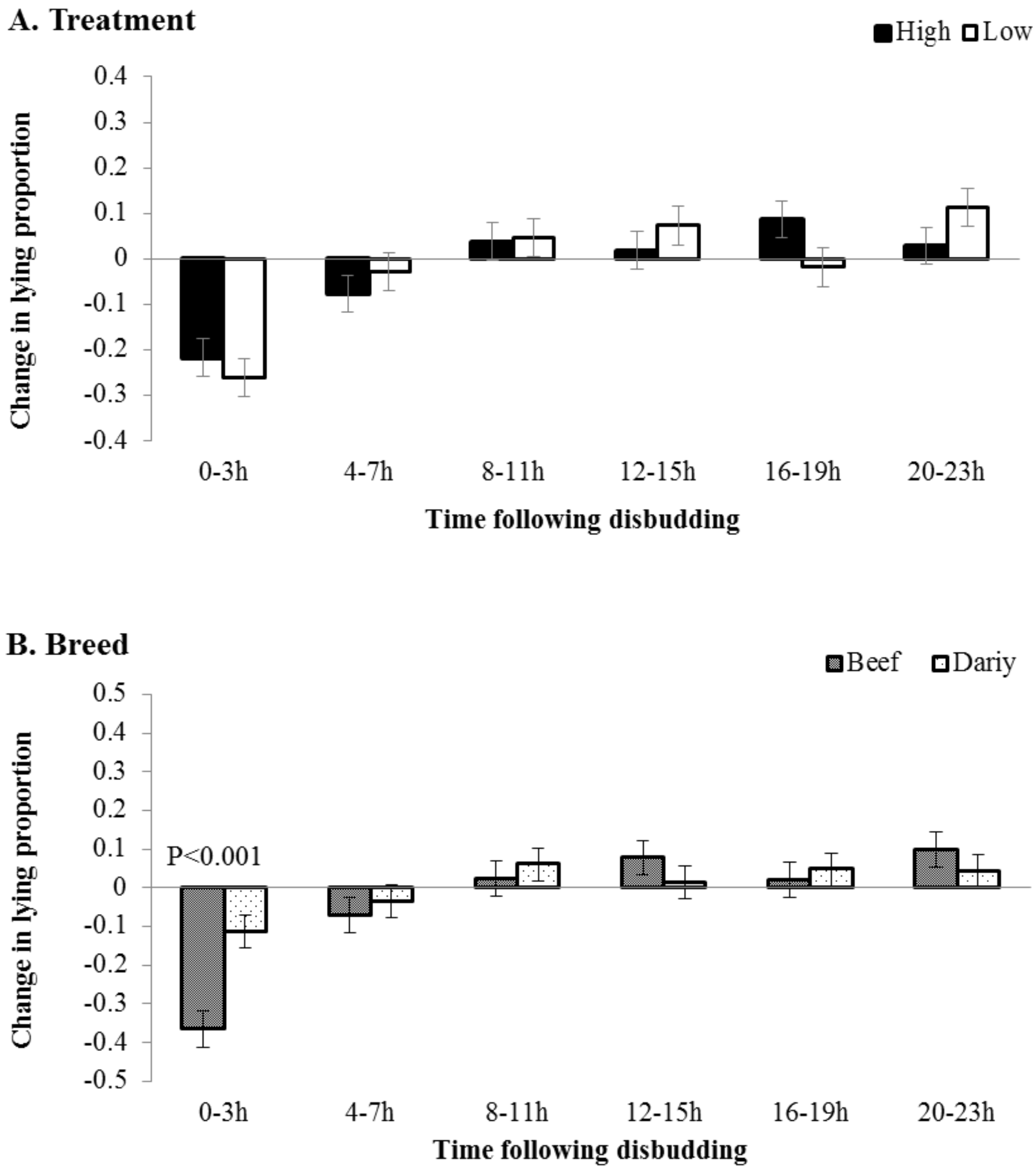

Figure 4.11. Change in lying proportion (mean±SEM) in each of the post-disbudding periods compared to the same period on the day before disbudding for each of the treatment groups (A) and breeds (B).

Levels of salivary cortisol before and after disbudding

There was no treatment effect on the salivary cortisol level at any sampling points ($F_{3,139.9}$=1.5, P=0.216). However, a significant effect of timing was observed ($F_{3,146.0}$=3.2, P<0.001), where the concentration of salivary cortisol significantly increased from the baseline (-24h pre-disbudding) to 0.5h post-disbudding (t=2.7, P=0.008), and gradually decreased at 4 h post-disbudding (**Figure 4.12**). Moreover, the increase in salivary cortisol levels from the baseline to 0.5h post-disbudding was statistically significant in the L group (t=2.0, P=0.044), although this was not significant in the H group (t=2.0, P=0.052). The level of cortisol returned to the baseline at 4h post-disbudding in the L group, and decreased further to a level lower than the baseline at 8h post-disbudding (t=2.2, P=0.027). In contrast, the level of cortisol in the H group remained above the baseline level at 4h post-disbudding and returned to the baseline at 8h post-disbudding. There was also a significant interaction between breed and timing on the salivary cortisol level ($F_{3,143.0}$=4.3, P=0.006). Dairy calves decreased their cortisol level to the baseline at 4h post-disbudding, whilst the salivary cortisol level for beef-cross calves remained higher until 8h post-disbudding (this was not statistically significant: **Figure 4.12**).

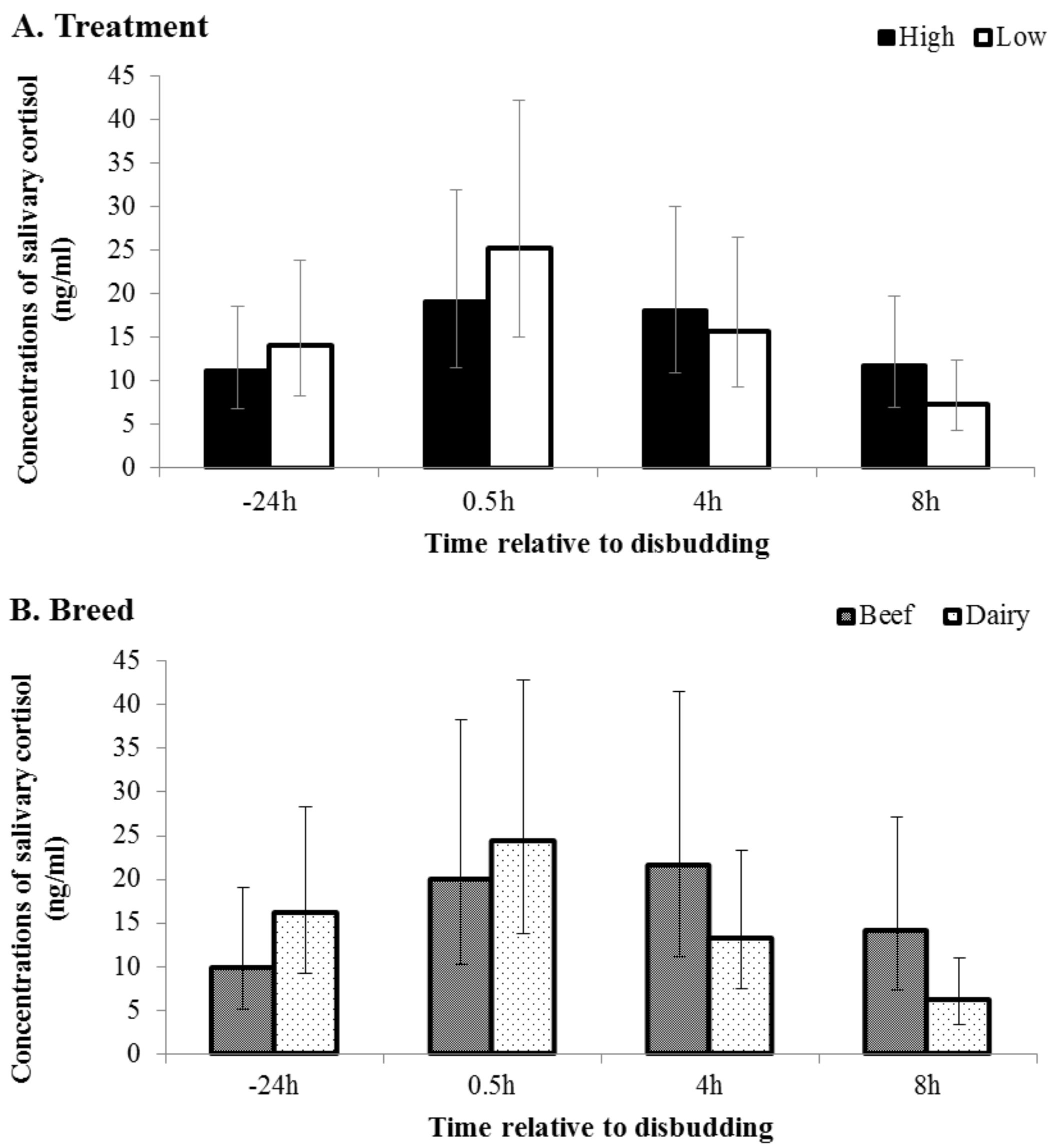

Figure 4.12. Change in the concentrations of salivary cortisol from baseline (24h pre-disbudding) to 0.5h, 4h and 8h post-disbudding. Effects of treatment (A) and breed (B) are presented as back-transformed means and corresponding 95% CIs.

4.4. Discussion

H calves were born to cows that experienced higher stocking conditions during the dry period (both in terms of lying area and feed-face: see **Chapter 3**). This dry period treatment resulted in cows taking longer to start feeding on fresh rations, being involved in more frequent competition at the feed-face, standing longer in the feed alley and spending less time feeding during the peak feeding period compared to cows in the low stocking density group. Previous studies have reported that restricted access to the feed-face and the lying area were associated with elevated levels of faecal glucocorticoid metabolites (Huzzey et al., 2012) or plasma cortisol (Fustini et al., 2017), suggesting that increased competition at the feed-face might have acted as a stressor for cows (Huzzey et al., 2012).

In the current experiment, however, there was no evidence that a high stocking density during the dry period induced a detectable physiological stress response in dry cows during late pregnancy. Therefore, it is potentially not surprising that only limited effects of maternal treatment were observed in offspring in the current study. However, the current study suggests that there may be some potential associations between maternal conditions during the prenatal period and particular behaviours in their calves, and this area is worth further investigation.

4.4.1. Body weight, health and growth

There was no effect of maternal treatment on the body weight of calves at birth, at d7 and at weaning, or on calf growth rate. In rodent models, maternal stress during pregnancy is associated with smaller litter size and/or lower birth weight of pups (Brunton, 2013). Moreover, some studies on other farm animals have found an association between maternal treatment during pregnancy and offspring body weight. For example, Corner et al. (2007) and Sphor et al. (2011) reported that shearing pregnant ewes increased the birth weight of their lambs. Jarvis et al. (2006) reported that maternal social mixing during pregnancy decreased the body weight of piglets at 35 days of age, whilst Roussel-Huchette et al. (2008) reported that maternal social isolation increased the body weight of lambs at 3 months of age. In contrast, the current study is in agreement with previous studies on sheep and pigs, where repeated transportation and social isolation (sheep: Roussel-Huchette et al., 2008), restraint or social mixing (pigs: Couret et al., 2009a; 2009b) experienced by dams did not affect the body weight of their offspring.

Calf birth weight can be influenced by various maternal factors such as age, milk production, parity and the length of the gestation period (Kamal et al., 2014). Lower birth weight of calves is associated with lower energy intake (Gao et al., 2012), heat stress (Tao et al., 2012a) and parasite infection (Loyacano et al., 2002) of cows during pregnancy. It has been reported that maternal heat stress (Tao et al., 2012a) and maternal undernutrition (Gao et al., 2012) can affect the immune function of offspring. However, no effect of maternal treatment during the prenatal period on serum IgG level at d7 or disease incidence in the pre-weaning period was observed in the current study.

4.4.2. Vigour of calves during the neonatal period and the first week of life

The current study did not find any evidence that maternal stocking density during the dry period affected the vigour of neonatal calves. There was a breed effect on the likelihood of calves moving to a sternal position, but no other behaviour was affected by breed. Early onset of neonatal behaviour such as standing and udder seeking enhances the early ingestion of colostrum. The adequate ingestion of sufficient amounts of colostrum quickly after birth (i.e. passive transfer of immunity) is essential for survival (Dwyer, 2003; Beam et al., 2009; Waldner and Rosengren, 2009). Campler et al. (2015) found an association between early onset of standing behaviour and early suckling in neonatal dairy calves. High vigour at birth is therefore advantageous to neonatal survival.

The current study found that assisted calving delayed newborn calves achieving a sternal position, but did not significantly affect the onset of other neonatal behaviours. It has been reported that dystocia delays the occurrence of some neonatal behaviours (Barrier et al., 2012) and hence is associated with increased pre-weaning mortality (Riley et al., 2004). The discrepancy between the current study and Barrier et al., (2012) may be partly due to slightly different definitions of neonatal behaviours, and the different statistical methods conducted. The current study compared the likelihood of a particular behaviour to occur, instead of comparing the actual time taken to perform each of the behaviours. In the dairy industry, colostrum is commonly administered via an oesophageal tube. The current study found that artificial colostrum feeding delayed the onset of standing and udder seeking behaviour. Calves' motivation to stand up and suckle (i.e. feeding motivation) may, therefore, be reduced by artificial colostrum feeding.

4.4.3. Activity level

Calves in the current study spent approximately 85% of the day lying in the first week, which is similar to the lying proportion found by Borderas et al. (2009a). Daily lying proportion of calves in the first week of life gradually decreased over time. In the current study, no significant treatment effect was observed on the lying proportion in the first week of life, and disease incidence in the hutch was the only significant factor affecting the vigour of calves. Calves treated for respiratory disease and/or diarrhoea spent almost 90% of the day lying, as opposed to 82% for healthy calves.

When calves were moved to a group pen, both groups reduced their lying proportion on their first days in the group pen compared to their lying proportion in the hutch, probably because they engaged in locomotor activities such as running and exploring. High motivation for locomotor behaviours has been reported in dairy calves after deprivation of locomotor behaviour due to confinement (Jensen, 1999, 2001). The effect of treatment was greater on the first day (a greater difference between means) than on the second day. The current study did not measure details of the lying proportion (e.g. lying bout frequency and duration), and so it is difficult to interpret the results in the context of other studies. Moreover, calves were introduced not only to a large enclosure but also to other calf companions. Therefore, fear of a novel environment and/or novel companions may have affected their activity level, in addition to their motivation for locomotor behaviour.

4.4.4. Training of the use of an automatic milk feeder

The ease of training scores (the "willingness of calves to enter the feeder" score and the "ease of finding a teat" score) can be used to assess the reactions of calves to human handling, and the calf's adaptability to a new feeding system. Associations between maternal stress and increased fear and anxiety-related behaviour in offspring have been reported in rodents and some farm animals (Weinstock, 2008; Otten et al., 2015). Therefore, it was hypothesised that H calves would potentially be more fearful and reactive to human handling, and might be more reluctant to enter the feeder and struggle to find the teat. The current study did not find any evidence that the maternal treatment affected these ease of training scores. The majority of calves (regardless of treatment group) hesitated to enter the feeder and required pushing from behind. In contrast, the majority of calves successfully found a teat by following the trainer's hand, with two calves in the H group finding a teat on their own.

Effect of maternal treatment was also not observed on the training count. The training count was used to assess not only the calf's ability to learn to use the automatic milk feeder, but also its adaptability to a novel environment with novel companions and a novel feed source. Maternal exposure to high stocking densities did not appear to have any effect on the calves' adaptability to the novel feeding system. However, the current study found that the training count was negatively correlated with the average daily weight gain in the group pen and in the pre-weaning period. Delayed self-feeding from the automatic milk feeder is associated with reduced milk intake over 1-2 weeks (Jensen, 2007; Fujiwara et al., 2014). These studies did not find any effect of delayed self-feeding on calf growth, probably due to the use of different training criteria than those used in the current study. The previous studies trained calves until they drunk half of the daily milk allowance (6.0 L: Fujiwara et al., 2014, 2.6/3.2 L: Jensen, 2007) whilst calves in the current study were trained twice per day, meaning that calves could consume up to 2.0 L of milk per day until they had learnt to self-feed. Delayed self-feeding from the automatic milk feeder can be a serious welfare problem and detrimental to calf growth.

4.4.5. Reactions to a group environment

The current study investigated whether maternal treatment would affect the behavioural reactions of calves to a novel environment with novel companions. Calves started walking, exploring and sniffing/licking companions within 30 seconds after the start of the observation period, when the calf came out of the milk feeder after the first training session. Regardless of maternal treatment, calves spent almost one-quarter of the observation period standing inactive. The duration of being inactive in an open-field test, the latency to approach the novel object, and the frequency of sniffing of the object (novel object test) are often used to assess fear in dairy cows (Boissy and Bouissou, 1995; Van Reenen et al., 2004). Longer durations of immobility, longer latency to approach the object and less frequent interactions with the novel object are associated with fear (Boissy and Bouissou, 1995; Van Reenen et al., 2004). H calves tended to take longer to start exploring the group pen (i.e. sniffing/licking objects) compared to L calves, which may reflect the higher lying proportion on the first day in the group pen. Lower locomotor activity is associated with fearfulness of dairy cows in a fear-eliciting situation (Boissy and Bouissou, 1995; Jensen et al., 1997), suggesting that H calves might be more fearful in a novel environment with novel companions.

L calves tended to receive social contact from their companions more frequently than H calves during the 30 minutes of the observation period. Dairy calves are motivated to socialise (Holm et al., 2002), and start interacting with other calves from two days of age (Duve and Jensen, 2012). However, there was no significant maternal treatment effect on social behaviours, including the duration of social contact, the latencies to initiate social contact, and the frequency of social contact initiated. Therefore, the motivation for calves to socialise may not be affected by maternal treatment, and the tendency for L calves to accept more frequent contact from companions might indicate that L calves were less fearful of unfamiliar companions. However, this requires further investigation. In contrast, some sheep studies (Roussel et al., 2004; Roussel-Huchette et al., 2008) have shown that lambs born to ewes that experienced social isolation and/or repeated transportation during pregnancy were less fearful in fear-eliciting situations compared to the control group (no prepartum stress). However, the current study indicated that prenatal exposure to maternal overstocking did not reduce fearfulness in dairy calves. Overall, maternal treatment did not have any significant effect on behavioural responses to the social housing system.

There was a significant interaction between treatment and group size in the duration of walking and running behaviours, with a greater treatment effect when the group size was larger, and H calves engaged in more walking and running behaviours as the group size increased. This may suggest that the locomotor behaviours of H calves were facilitated by an increased number of companions. Another hypothesis is that L calves became inactive with limited space allowance due to an increased number of companions. In the current experiment, the effects of greater space allowance and the presence of companions on the reactions of calves to a group environment were observed simultaneously. This is because the main aim of this experimental work was to assess the reactions of calves to a group housing system, a situation that calves often experience on commercial farms.

Age at introduction affected the locomotor behaviour of calves and the duration of social interactions. Calves introduced at d7 took longer to start walking and running, and spent longer periods of time inactive compared to calves introduced at d8. However, calves introduced at d7 had more frequent social contact with companions and for longer periods of time compared to calves introduced on d8. This may suggest that younger calves were more fearful of a novel environment, but less fearful of their novel companions. It has been reported that calves introduced to the group environment on d6 tended to spend less time standing, and made less social contact compared to calves introduced on d14 (Rasmussen et al., 2006). There were only six calves that were introduced into the group pen on d8, compared to 29 calves that were introduced on d7. It is uncertain whether only one day of age difference could make such an impact on calves' social motivation, and thus this finding requires further investigation with a larger sample size.

Disease incidence in the hutch had a significant effect on the activity levels and social behaviour of calves during the first 30 minutes in the group pen. Calves treated for illness in the hutch were quicker to lie down and made less social contact with companions. This suggests that calves that were sick in the hutch were less active when introduced to a group environment and showed less social motivation towards companions. Calves that were sick in the hutch also showed higher lying proportions on the first days in the group pen, which confirms their lower activity level.

4.4.6. Behaviour in the first two weeks in the group pen

Activity level, feed-related behaviour and social behaviour of calves were investigated on four different occasions in the first two weeks in the group pen. Although there was no significant treatment difference in the daily lying proportion, L calves spent 74% of the observation period lying whilst H calves spent 67% of the day lying. A positive correlation between lying time and daily weight gain has been reported in dairy calves and heifers (Mogensen et al., 1997; Hänninen et al., 2005), suggesting the importance of adequate resting. However, slightly higher lying proportion of L calves was not reflected in their growth in the group pen. Calves had the highest lying proportion in the summer (July and August) compared to winter and spring (from January to June). It has been reported that heifers increased their lying duration during periods with lower temperatures and lower solar radiation (Redbo et al., 2001). However, it has also been reported that cows spent less time lying on a wet surface (Keys et al., 1976; Fregonesi et al., 2007b). In Scotland, winter is likely to be wet and windy compared to summer, and the calf barn used for this experiment had no barriers to block the ingress of wind and rain. The surface of the straw yards could potentially be wet when it was raining, which may explain the lower lying proportion of calves in winter compared to summer. However, the usage of straw yards and igloo sheds was not affected by season.

H calves initiated social contact with companions more frequently than L calves. L calves were more likely to receive social contact from companions compared to H calves, but this was only when the group size was larger. A calf's social motivation markedly increases between two to three weeks of age (Vitale et al., 1986; Duve and Jensen, 2012), which corresponds to the observation period of the current study. Early social experience is essential for the development of social behaviour (de Paula Vieira et al., 2012b), and is associated with improved cognitive abilities (Gaillard et al., 2014) and social competency (Buchli et al., 2017). It appears that H calves were more motivated to socialise with companions, which would be advantageous for calves in group housing environments.

There are other methods used to assess sociability and social ability in dairy cattle. For example, Holm et al. (2002) measured the motivation of calves to have limited or full social contact in an operant conditioning test. Gibbons et al. (2010) investigated relationships between the motivation to be closer to pen mates, and behavioural synchrony or proximity to other cows. In the current study, the proximity to other calves was the only feasible measure available to investigate the social behaviour of calves in addition to social contact. However, this study found no treatment effect on the proximity of calves to neighbouring calves. In laboratory rodent models, maternal stress has been reported to impair the social behaviour of offspring, but animals were often tested in aversive conditions (e.g. exposure to novelty and social isolation, reviewed by Braastad, 1998). In the current study, the effect of maternal treatment on the social behaviour of calves was not consistent, probably because the experimental setting for the calves were not environmentally challenging.

The frequencies of milk and starter feeder visits were observed in order to investigate the effect of treatment on feeding behaviour of calves. In the current study, the milk allowance per visit was restricted to 1.0 L, which is much smaller than the amount fed in the hutch (3.0 L) or the amount that calves could normally consume as one meal (4.7 L: Appleby et al., 2001). With automatic milk feeding systems, it has been reported that calves that were fed restricted amounts of milk made more frequent visits to the feeder, even when calves were not entitled to drink milk (de Paula Vieira et al., 2008; Jensen and Holm, 2003; Jensen, 2006; Nielsen et al., 2008), which reduces the efficiency of the feeder. On the other hand, calves increase solid feed intake after two weeks of age when the milk allowance is restricted (e.g. to 10% of the calf body weight: Jasper and Weary, 2002). In young ruminants, solid feed intake is also enhanced by social companions (de Paula Vieira et al., 2010, 2012a). Therefore, the current study hypothesised that maternal treatment might affect calf reactions to restricted milk feeding and initiation of starter intake, but found no evidence that maternal treatment affected feeding behaviour.

4.4.7. Behaviour around weaning

Weaning is one of the stressful procedures experienced by farm animals, as it usually occurs at an earlier age compared to natural conditions (Dybkjær, 2008; Weary et al., 2008). In cattle, the weaning process may be psychologically less stressful compared to other farm animal species, as it does not involve any separation from the dam. In the current study, calves were weaned off milk over 10 days, which is supposed to be less stressful compared to abrupt weaning (Nielsen et al., 2008).

However, some behavioural responses to the gradual weaning process were observed. Although there was no significant treatment effect, calves from both treatment groups increased the frequency of milk feeder visits from an average of two to five times per observation. It has been reported that the frequency and the duration of unrewarded visits (a visit to the feeder when calves are not entitled to drink milk) increased during gradual weaning (Nielsen et al., 2008). The current study was unable to distinguish between rewarded and unrewarded visits, but theoretically calves were entitled to drink milk in up to two visits within the observation period (lasting 180 min). This means that calves made on average three unrewarded visits per observation during weaning. The frequency of starter feeder visits also increased after the weaning process started, suggesting that calves increased their starter intake during gradual weaning, a response which has been found in previous studies (Jasper et al., 2008; Nielsen et al., 2008).

A significant maternal treatment effect was also found in the lying proportion. Before weaning started, the lying proportion was not different between the maternal treatment groups. However, the lying proportion for H calves decreased significantly after the gradual weaning process had started, whilst the lying proportion for L calves remained constant. When the weaning process was completed, the lying proportion for H calves returned to the same level as the day before weaning. It has been reported that calves stood up more frequently and spent more time standing during weaning (Budzynska and Weary, 2008). Therefore, the reduced lying proportion in H calves may indicate that H calves became more restless during gradual weaning. Similarly, Black et al. (2017) reported that maternal grazing during late pregnancy resulted in shorter lying times in offspring during weaning compared to maternal confinement, indicating a potential effect of maternal treatment on offspring reaction to weaning.

Furthermore, the current study found a significant treatment effect on the location of calves. Both H and L calves were more often observed in the straw yard in the middle of weaning compared to the day before weaning. This is potentially because all the feeders were located in the straw yard, and calves may have wanted to stay closer to the feeders. However, H calves were still more likely to be in the straw yard when the weaning process was completed, whilst the location of L calves returned to the same pattern as the day before weaning. These results suggest that maternal treatment may have affected offspring behavioural reactions to weaning.

4.4.8. Reactions to disbudding

Disbudding or dehorning is a common husbandry practice on cattle farms (Stafford and Mellor, 2005) as hornless animals are easier to handle, reduce the risk of injury, and require less feed-face (Stookey and Goonewardene, 1996). The heads of calves have to be manually restrained with a head bail during cautery disbudding as this procedure is considered painful (Stafford and Mellor, 2011). The current study found that any maternal treatment effect on the reactions to cautery disbudding was exerted differently between calves that were previously sick in the group pen and calves that were not sick in the group pen. If calves were not sick in the group pen, L calves showed more frequent head moving and kicking during disbudding, compared to H calves. In contrast, if calves had been sick in the group pen before the day of disbudding, there was no treatment effect on the behavioural reactions during disbudding. Rapid movement of head and legs observed during the disbudding procedure are associated with acute responses to stressful procedures and pain (Graf and Senn, 1999; Grøndahl-Nielsen et al., 1999). Although local anaesthesia can effectively reduce the pain during disbudding, previous studies have shown that calves still displayed some behavioural reactions to disbudding (Graf and Senn, 1999; Grøndahl-Nielsen et al., 1999). Therefore, it is possible that calves felt some degree of pain or discomfort during disbudding, and maternal high stocking density treatment reduced the reactivity of healthy calves to disbudding.

Behaviours such as head shaking, ear flicking and head rubbing after disbudding are considered indicators of pain (Graf and Senn, 1999; Faulkner and Weary, 2000; Heinrich et al., 2010). The current study observed pain-related behaviours at 3.5h post-disbudding, when the effect of local anaesthesia started to wear off (Faulkner and Weary, 2000), and at 6h post-disbudding when the effect of local anaesthesia was predicted to have disappeared. At 3.5h post-disbudding, a significant effect of maternal treatment was observed on the reactions of calves to pain, when calves were not sick in the group pen. The maternal high stocking treatment reduced the exhibition of pain-related behaviour in healthy calves, but this maternal treatment effect was not observed in calves that were sick in the group pen.

Previous disease incidence appeared to inhibit L calves in their expression of pain-related behaviours, but this effect was not observed in H calves. It has been reported that calves in a state of ill-health showed reduced expression of some behaviours such as exploration and social behaviours (Weary et al., 2009; Cramer and Stanton, 2015). Together with the results of behavioural reactions to disbudding, it appears that maternal high stocking density treatment suppressed the reactivity of healthy calves to painful procedures and the expression of pain-related behaviour. In contrast, Sandercock et al. (2011) reported that prenatal stress reduced the sensitivity to noxious and cold stimulation in piglets. Therefore, from the results of the current study, it could be argued that the maternal high stocking treatment resulted in a reduced sensitivity of healthy calves to pain. To understand the mechanisms of the relationship between maternal treatment and offspring reactions to pain would require further research.

Regardless of the maternal treatment groups, the level of cortisol in saliva was significantly elevated at 0.5h post-disbudding compared to the baseline period. Elevation of plasma cortisol levels after cautery disbudding has been reported in previous studies, even after LA administration (Petrie et al., 1996; Graf and Senn, 1999; Heinrich et al., 2009). In cattle, significant positive correlations were reported between concentrations of plasma and salivary cortisol in response to the activation of the hypothalamic-pituitary-adrenal (HPA) axis (Negrão et al., 2004; Pérez et al., 2004). In the current study, L calves showed a more distinct increase in the concentration of salivary cortisol at 0.5h post-disbudding compared to H calves, suggesting that L calves may have a higher responsiveness of the HPA axis compared to H calves. Higher responsiveness of the HPA axis has been reported in the offspring of various animal species that were exposed to higher glucocorticoid levels during the prenatal period (Haussmann et al., 2000; Jarvis et al., 2006; Brunton and Russell, 2010). Rutherford et al. (2009) also reported that prenatal exposure to higher maternal cortisol resulted in higher pain scores of a litter of piglets to a painful procedure (tail-docking).

In the current study, concentrations of maternal faecal glucocorticoid metabolites were not significantly different between the maternal treatment groups, although L cows showed higher concentrations compared to H cows, especially in the beginning of the dry period (see **Chapter 3**). This may potentially explain the slightly more distinct responses of the HPA axis in L calves to painful procedures, and possibly the more frequent pain-related behaviours in the L group. Both cows and calves showed large individual animal variations in their levels of faecal glucocorticoid metabolites or salivary cortisol. Therefore, any investigation of relationships between maternal and offspring cortisol levels would allow for a better understanding of the effect of prenatal environment on the behavioural and physiological reactivity of their offspring to pain.

Lying proportion following disbudding decreased from the baseline period (24 hours before) for both treatment groups. Increased activity and restlessness have been reported in calves that were disbudded without local anaesthesia (Morisse et al., 1995) or without non-steroidal anti-inflammatory drugs (Heinrich et al., 2010). However, no effect of maternal treatment was observed in the current study, and the lying proportion returned to the same level as the day before at 4-7h post-disbudding.

4.4.9. Effects of other variables of interest on offspring performance

It has been reported in other species that there are breed and gender differences in the outcomes of maternal stress (Dwyer, 2003; Brunton and Russell, 2010). However, the current study rarely observed interactions between breed or gender and maternal treatment, probably due to a relatively small sample size. The effect of disease incidence was observed in the calf growth rate, weaning weight and activity levels. Moreover, disease incidence affected the social behaviour and pain-related behaviour of calves. Dairy calves are normally separated from their dams immediately after birth and artificially reared by humans. Therefore, it is possible that the growth and health status of calves are more likely to be influenced by calf management rather than prenatal experience, highlighting the importance of disease control in the calf.

4.4.10. Overall effect of maternal treatment on offspring performance

A number of studies in laboratory animals suggest that maternal stress can affect fetal brain development, altering stress reactivity in the offspring (reviewed by Charil et al., 2010). The regions of the brain associated with cognitive ability and emotions are shown to be susceptible to maternal stress (Weinstock, 2008; Charil et al., 2010). In farm animal species, studies in pigs found that maternal social stress and the associated increase in circulating cortisol levels altered the development of brain regions that are related to fear and anxiety (Otten et al., 2015). Therefore, excessive secretion of cortisol due to higher levels of stress during pregnancy could result in cognitive and behavioural problems in offspring. In the current study, however, there was no significant treatment effect on the concentrations of faecal glucocorticoid metabolites in cows, suggesting that the circulating cortisol levels of cows in the high stocking density group were potentially not high enough to affect the programming of the fetal brains. This could explain the lack of measurable significant effects of maternal treatment on most of the calf outcomes measured in this study.

Moreover, there were large individual animal variations in maternal faecal glucocorticoid levels and behavioural outcomes of the calves. Therefore, it is possible that some individual cows in the low stocking density group had higher stress levels than individual cows in the high stocking density group, and their offspring were affected accordingly. Nevertheless, the current study indicated that there were some detectable effects of maternal high stocking density treatment on calf social behaviour, activity level and reactivity to pain and weaning. These behavioural outcomes may not necessarily be mediated by maternal cortisol levels, and may not necessarily be considered negative or harmful. Investigations of individual cow-calf relationships may be able to find associations between prenatal experience and calf outcomes, which may have been masked by the wider group comparisons.

4.5. Conclusions

The current study indicates that prenatal exposure to maternal high stocking density during late pregnancy had no detectable impact on calf birth weight, growth rate during the pre-weaning period, or the vigour of calves in the neonatal period and in the first week of life. The calf growth rate during the pre-weaning period and activity levels in the first week of life were more likely to be affected by disease incidence rather than prenatal maternal experience, highlighting the importance of disease control in pre-weaned calves. Disease incidence also affected social behaviour and pain-related behaviour of calves.

Maternal high stocking treatment did not affect the learning ability of calves, measured by their training in the use of the automatic milk feeder. No maternal treatment effect was observed in the willingness of calves to enter the feeder or a crush, suggesting that reactions to human handling were also not affected by maternal treatment. Behavioural responses to a group environment suggested that L calves may be less fearful of a novel environment and novel companions. L calves spent more time resting in the group pen, which may be advantageous for calf growth. H calves initiated more social contact, probably because they were more motivated to socialise, which would be advantageous for group housing.

At weaning, H calves decreased their lying proportion and spent more time closer to the feeders, which may suggest that H calves showed higher reactivity to weaning compared to L calves. In contrast, behavioural reactions to disbudding were more distinct in L calves, showing more frequent pain-related behaviours and a significant increase in salivary cortisol levels after disbudding. This treatment effect on the behavioural responses of L calves to pain was mainly observed in healthy calves. These outcomes were not consistent and it is not possible to draw any conclusions that maternal high stocking density during pregnancy was either advantageous or disadvantageous for offspring. Further research on individual maternal stress levels and their association with offspring stress levels may be helpful in order to assess the behavioural outcomes in offspring. The interactive influence of maternal treatment and offspring health status in expression of responses to painful procedure would also be worth further investigation.

Chapter 5 :

Effects of out-wintering pregnant heifers on calf growth and behaviour

5.1. Introduction

Traditional farming systems in the United Kingdom normally keep cows outside during the summer, and keep cows indoors during the winter. During the winter housing period, cows are typically fed a mixed ration in a group housed shed. In such herds, heifers may also be kept indoors in winter. However, due to the reduced management costs and potential welfare benefits compared with traditional indoor winter housing, there is a growing interest in rearing replacement dairy heifers outdoors in winter as an alternative to a confinement system. Studies have shown that cows coped well with winter weather where shelters and a dry lying area were provided (Redbo et al., 2001, Tucker et al., 2007). O'Driscoll et al. (2010) reported that out-wintering of dry cows did not compromise milk production during the subsequent lactation period. In heifers, higher incidences of comfort behaviour (e.g. self-grooming), social grooming and smelling, and play behaviour were observed in heifers out-wintered on wood-chip pads compared to indoor heifers (Boyle et al., 2008).

In contrast, Boyle et al. (2008) found that out-wintered heifers had reduced dry matter intakes and weight gain compared to indoor heifers. Reduced feeding time and compromised metabolic status were also reported as a consequence of out-wintering in harsh weather conditions (Redbo et al., 2001; Tucker et al., 2007; Webster et al., 2008). Keogh et al. (2009) also reported that out-wintering on perennial ryegrass decreased body condition of prepartum beef cows, while this effect was not observed in cows offered perennial ryegrass silage either indoors or outdoors with kale or swede. Heifers may be more prone to cold stress due to a larger body surface to weight ratio compared with adult cows (Berman, 2003). Harsh winter conditions (e.g. wet and windy weather) have been reported to reduce time spent lying and increase levels of plasma cortisol/faecal cortisol metabolites (Tucker et al., 2007, Webster et al., 2008), which are indicative of poor welfare.

If animals were exposed to such harsh weather conditions during pregnancy, it could affect fetal development. It has been reported in various mammalian species that maternal stress during pregnancy can have a detrimental effect on offspring development, which leads to life-long impacts on health, behaviour and cognitive abilities (e.g. Arnott et al., 2012, Braastad, 1998). In dairy cows, heat stress during late pregnancy has been reported to affect offspring immune function, although it did not affect calf growth (Tao et al., 2012a).

Associations between insufficient maternal nutrition and impaired fetal growth and postnatal performance have also been reported in beef cattle (reviewed by Wu et al., 2006; Funston et al., 2010a). For example, Martin et al. (2007) reported that out-wintering of cows on pasture with no protein supplementation during the last trimester of gestation resulted in lower weaning weights and productivity of offspring. In dairy cows, Gao et al. (2012) reported that energy restriction of prepartum cows resulted in smaller body size and impaired immunity in neonatal calves. These studies suggest that offspring health and growth can be influenced by maternal nutrition during the prenatal period. However, the effects of prenatal exposure to maternal out-wintering on fetal development and postnatal behaviour in offspring have not been investigated.

To study these effects, this study followed spring-born calves born to heifers either kept indoors and fed grass silage, or outdoors and fed silage and grazing on either kale or deferred grass to investigate whether maternal exposure to winter conditions during pregnancy would negatively or positively affect fetal development and behavioural responses to challenges in the first two weeks of life. Parameters measured included calf body weight, growth and activity levels of calves, and learning abilities and behaviours of calves in a group housing system.

5.2. Materials and methods

The experiment was conducted at the Crichton Royal Farm SRUC in the Dairy Research and Innovation Centre (Dumfries, UK). The experiment was approved by the SRUC Animal Welfare and Ethical Review Body (Animal Experiment Number: AE11-2015).

5.2.1. Heifer feeding and housing management

In November 2014, 48 Holstein Friesian pregnant heifers (23±0.3SD month old) that were expected to calve between mid-February and mid-April 2015 were enrolled in the out-wintering project. Heifers were balanced to treatment group by body weight and expected calving date, and allocated to one of three treatments. They spent the whole winter period either in 1) an indoor cubicle shed (36m×6m: I group), 2) out-wintered on deferred grass grazing (106m×363m: G group), or 3) out-wintered on growing kale (100m×301m: K group). The deferred grazing system utilised the grass sward that was allowed to grow in the late summer to allow grazing throughout the winter (Hargreaves et al. 2016, unpublished). I heifers were offered a total mixed ration delivered once a day, and both G and K heifers were offered grass silage *ad libitum*. Minerals were supplemented to all groups through mineral licks. The heifers remained in their respective treatment groups until approximately 4 weeks before their expected calving date. They were then moved to a straw-bedded shed with dry cows and fed a dry cow total mixed ration until calving. Water was constantly available from a large trough on the field for the G and K groups and in the shed for the I group.

5.2.2. Calf feeding and housing management

Thirty-five spring born calves (born between 23rd February and 31st May 2015) were monitored until 14 days of age (I: n=10, K: n=11, G: n=14). The majority of calves were beef-cross breed (Holstein Friesian×Aberdeen Angus: n=22, Holsten Friesian×British Blue: n=7), and six calves were dairy breed (Holsten Friesian). Distributions of gender and breed (beef-cross or dairy) for each treatment group are summarised in **Table 5.1**. Irrespective of maternal treatments, all calves were managed in the same way. Four litres of colostrum was fed with a stomach tube within four hours after birth. Calves were then weighed (BW birth) and moved to an individual hutch (1.0 × 1.4m × 1.0m) with a straw-bedded front yard (1.2m×1.4m). Calves were fed three litres of milk twice a day (8:00 and 15:30) from a bucket with a rubber teat. Details of feeding management are described in **Chapter 4, Section 4.2.1** (**p181**). Due to a change in calf feeding management on the farm from March 2015, 32 calves were fed non-pasteurised whole milk, and three calves were fed milk replacer. Day1 (d1) was defined as when calves first received milk other than colostrum in the hutch, and calves stayed in the hutch until d7±1.

Table 5.1. Distributions of gender and breed of calves in each of the treatment groups.

	Bull		**Heifer**	
	Beef-cross	**Dairy**	**Beef-cross**	**Dairy**
Indoor (I)	3	2	5	0
Outdoor on Grass (G)	5	0	7	2
Outdoor on Kale (K)	5	1	4	1

At d7±1, calves were weighed (BW introduction) and moved to a straw-bedded group pen (7.5m × 5.0m) with access to an igloo shed (5.0m × 5.0m × 1.5m) at the end of the pen (see Figure 1 in Chapter 4). Calves were moved before a morning feeding (between 7:30 and 10:30) and were then trained to feed from an automatic milk feeder. A training protocol was created to ensure that all calves were taught in the same manner. Details of training and feeding management in the group pen are described in **Chapter 4, Section 4.2.2.4** (**p186**). Group structure was dynamic with calves entering and leaving depending on date of introduction from the hutches and subsequent weaning dates. Group size was from 2-15 calves per group, except for the period from 2nd to 18th of March 2015 when two group pens were combined due to a shortage of calf accommodation. The double-sized group pen had one milk feeder, two straw and starter concentrate troughs and two water cups with the maximum group size being 25. Calves were weighed at the end of the experiment (=14 days of age: BW d14).

Video cameras (Hi Res Bird Box Camera, 700TVL Sony EFFIO CCD, IR Night Vision, SpyCamera CCTV Ltd., Bristol, UK) were attached to the ceilings, columns of the calf barn and the ceilings of the igloo shed. Videos cameras were connected to a digital video surveillance system (GeoVision, version 8, GeoVision Inc., Taipei, Taiwan) that stored video footages from 06:00 to 18:00.

5.2.3. Data collection

5.2.3.1. Health and growth

The health condition of calves was monitored every day by farm staff, and any incidence of disease and associated treatments (veterinary or non-veterinary) were recorded. The average daily gain (ADG; g/day) in the hutch, in the group and in the first 14 days of life were calculated as follows:

- ADG hutch (g/day) = BW introduction – BW birth/(age at introduction – 1)
- ADG group (g/day) = BW d14 – BW introduction/(14 – age at introduction)
- ADG 14 days (g/day) = BW d14 – BW birth/14

5.2.3.2. Activity level

A data logger (AX3, Axivity, Newcastle Upon Tyne, UK) was attached to the hind leg of each calf from d1 to d9 to measure activity level. Details of data collection from the data logger are described in **Chapter 4, Section 4.2.2.4** (**p185**). Daily lying proportion in the hutch (LP hutch) and in the first two days in the group pen (LP group) was calculated using pivot tables in Excel 2013.

5.2.3.3. Training count and ease of learning scores

The number of training sessions required for calves to self-feed was recorded for each calf (training counts). Video clips of the first training were watched for all calves to evaluate how easily calves were trained in the first training. “Ease of training” scores (**Table 5.2**) were used to assess how willingly calves entered the feeder (“willingness to enter the feeder” score) and how easily calves found a teat (“ease of finding a teat” score).

Table 5.2. Description of the ease of training scores.

Willingness to enter the feeder	
0: No help	The calf enters the feeder on its own.
1: Easy	The calf enters the feeder by following the trainer's hand (no push).
2: Not Easy	The calf hesitates to enter the feeder so the trainer needs to gently push the calf from behind.
3: Difficult	The calf refuses to enter the feeder so the trainer holds its body and pushes into the feeder, or more than one trainer are needed.
Ease of finding a teat	
0: No help	The calf finds a teat on its own.
1: Easy	The calf finds a teat by following the trainer's hand.
2: Not easy	The trainer has to hold the head of the calf or guide it to the teat 2-3 times until the calf starts drinking.
3: Difficult	The calf does not keep sucking so the trainer must remain with the calf to repeatedly guide the calf to the teat until it consumes the whole portion. Sometimes two trainers are needed to keep the calf in the feeder.
4: Very difficult	The calf refuses to suck, so the trainer has to squeeze milk for the calf and hold its muzzle to ensure that the calf is drinking, and/or the calf does not consume the whole portion. Sometimes two trainers are needed to keep the calf in the feeder.

5.2.3.4. Reaction to a group environment

Reactions of calves to a novel environment with novel pen mates were continuously video observed by a single person for 30 minutes using Observer® XT 12.5 (Noldus Information Technology b.v., Wageningen, The Netherlands). The observation started when the calf finished its first meal (when the whole body of the calf was out of the feeder either on its own or being removed by the human trainer). Data collected included location in the group pen (milk feeder, straw yard, igloo shed), posture (lying, standing, walking, running, other), behaviour (exploratory, social, self-grooming and other) of the calf, and event or state (human intervention, social contact from pen mates, introduction of a new calf) were recorded. Details of the ethogram are shown in **Table 4.3** (**p189**)**.** Latencies of the calf to first perform each of the behaviours and to first enter the igloo shed, durations of each of the locations and postures, durations of each of the behaviours, and frequency of the behaviours and the event/state were calculated.

5.2.4. Data processing and statistical analysis

Statistical analyses were performed using Genstat® 16th Edition (VSN International Ltd, Hemel Hampstead, UK), and figures generated using Excel 2013. Test statistics, degrees of freedom (*df*), P-value, means or predicted means and standard errors of means (SEM) were reported. Individual statistical models were built for each of the variables measured, and the effect of treatment was tested after being adjusted for other variables of interest, such as calf characteristics (gender, breed), disease incidence (hutch, group pen, 14 days) and month of observation (from February to June). In addition to these variables, the type of milk fed (whole milk or milk replacer) was tested as univariate in the analyses for ADG in and LP in the hutch. Group size (covariate) was tested for the analyses of the training count and the behaviours in the group pen. These variables were first tested independently as univariates, and those with a significant level at P<0.25 were included with treatment in the final model. Interactions between treatment and variables of interest were fitted for all of the analyses by backward stepwise selection, except when using regression models. Normality of the residuals was checked graphically, and the data were transformed where necessary. Means obtained from transformed data were back-transformed and reported with 95% confidence intervals [95% CIs]. A post-hoc analysis (Fisher's unprotected least significant difference test) was conducted when there were significant interactions or significant differences between more than two categories (e.g. treatment) to investigate the direction of the effect.

BW and ADG were analysed using general linear models (GLM). The training count (covariate) was also tested as a univariate when analysing the ADG group and ADG 14 days. The effect of maternal treatment on the percentages of calves treated for illness was tested using Fisher's exact test. LP hutch and LP group were analysed by linear mixed model using residual maximum likelihood procedure (REML). The final mode for LP hutch included treatment and "day(s) in the hutch" as fixed effect together with other variables of interest (P<0.25 in the univariate analysis), and calf as a random effect. The final model for LP group included treatment and "day(s) in the group pen" (group d1: 0-24 hours, group d2: 24-48 hours) as fixed effects together with other variables of interest (P<0.25 in the univariate analysis), and calf and treatment nested within pen (pen/treatment) as random effects. The training count was analysed using REML, including pen/treatment as random effects. Ordinal logistic regression was used to analyse ease of training scores, with the final models being adjusted for the pen location.

Behavioural data in the first 30 minutes in the group pen were processed as follows. The events of "self-grooming", "no social contact" and "new calf introduction" were not included in the analyses because of very few observations. Sniffing/licking/nosing companion(s) (head, belly, other) were combined as “social contact initiated” because of very few observations of sniffing/licking belly and other parts of the body. Latencies of each calf to first perform walk, run, explore, enter the igloo shed, lie down, initiate social contact with pen mates, and receive social contact from pen mates (i.e. social sniffing/licking/nosing) were calculated. Time spent inside the igloo shed or in the straw yard (excluding the time spent in the milk feeder), time spent standing inactive or engaging in the following behaviours (walk, run, explore and social contact) were also calculated. Frequencies of walking, running, exploring, and social contact (initiated or received) were also calculated.

Latencies for each of the behaviours in the first 30 minutes in the group pen were analysed using proportional hazards (Cox) regression after being adjusted for the pen location. The likelihood of the behaviours occurring was indicated by a hazard ratio (HR) with 95% CIs. Durations and frequencies of each of the behaviours in the first 30 minutes in the group pen were analysed using REML, including treatment with the other variables of interest ($P<0.25$ in the univariate analysis) as fixed effects, and pen/treatment as random effects. The time spent in the igloo shed was analysed following a square root transformation. Additional variables of interest in the analyses of the behavioural reactions to a group environment included human presence inside or outside the pen during the observation period (yes, no).

5.3. Results

5.3.1. Body weight, health and growth

The final models for BW and ADG are shown in **Table 5.3**.

Table 5.3. Final models used to analyse BW and ADG.

	Final model
BW birth	*treatment + gender*
BW introduction	*treatment + gender*
BW d14	*treatment + gender + disease incidence (14 days)*
ADG hutch	*treatment + gender + disease incidence (hutch)*
ADG group	*treatment + gender + month*
ADG 14 days	*treatment + disease incidence (14 days) + training count + month*

Table 5.4 summarises BW and ADG for the three treatment groups. There were no significant differences between the three treatments in BW birth ($F_{2,31.0}$=1.7, P=0.201). Significant treatment effects were found in BW introduction ($F_{2,31.0}$=4.2, P=0.025) and BW d14 ($F_{2,30.0}$=4.4, P=0.020), where K calves were significantly heavier than G calves at introduction (t=2.9, P=0.007) and at d14 (t=3.0, P=0.006). BW introduction and BW d14 for I calves were not significantly different to G and K calves.

ADG hutch was not significantly different between treatment groups ($F_{2,30.0}$=0.5, P=0.619), but there was a large individual variation. On average, calves from all the three treatment groups lost body weight in the first week of life (**Table 5.4**). ADG group was not different between the treatment groups ($F_{2,28.0}$=1.2, P=0.325), but a significant treatment effect was found in ADG 14 days ($F_{2,27.0}$=7.2, P=0.008). ADG 14 days for G calves was significantly lower than I (t=3.5, P=0.002) and K calves (t=3.1, P=0.005), whilst there was no difference in ADG 14 days between I and K calves.

The percentages of calves treated for diarrhoea and/or pneumonia over the 14 days were 10.0% (1/10), 35.7% (5/14) and 27.3% (3/11) for I, G and K group, respectively. Fisher's exact test showed that there was no significant difference between the treatment groups in the disease incidence in the first 14 days of life (I vs G: P=0.341, I vs K: P=0.586, G vs K: P=0.653).

Table 5.4. Body weight (BW: kg) and average daily gain (ADG: g/day) of calves born to heifers kept indoors (Indoor), outdoor grazing on deferred grass (Grass) and outdoor grazing on kale (Kale) during the winter period.

	Indoor (I) n=10	**Grass (G) n=14**	**Kale (K) n=11**	**P-value**
Body weight (kg)				
birth	42.2±1.4	41.0±1.2	44.4±1.4	0.201
introduction	42.1±1.3^{ab}	39.7±1.1^{a}	44.6±1.3^{b}	**0.025**
d14	43.7±1.4^{ab}	41.1±1.1^{a}	45.9±1.3^{b}	**0.020**
Daily gain (g/day)				
hutch	-153.5±167.1	-298.3±132.7	-134.9±179.5	0.619
group	526.9±96.9	336.0±86.8	419.2±94.5	0.325
14 days	319.4±60.2^{a}	50.8±50.0^{b}	255.9±52.5^{a}	**0.003**

Different superscripts indicate significance levels at P<0.050.

There was a significant gender effect on BW birth (bulls: 45.1±1.1, heifers: 40.0±1.1 kg, $F_{1,31.0}$=11.1, P=0.002), BW introduction (bulls: 45.3±1.1, heifers: 38.9±1.0 kg; $F_{1,31.0}$=20.0, P<0.001) and BW d14 (bulls: 46.5±1.0, heifers: 40.6±1.1kg, $F_{1,30.0}$=18.3, P<0.001). In the first week of life, the average body weight loss in heifer calves was approximately 5.4 times greater than in bull calves (bulls: -61.3±130.7 g/day, heifers: -329.9±153.8 g/day), although this was not statistically significant ($F_{1,30.0}$=3.1, P=0.089). In contrast, the gender difference in the ADG was smaller in the group pen (bulls: 343.6±80.8, heifers: 511.2±73.9 g/day, $F_{1,28.0}$=2.7, P=0.111). This resulted in no difference in ADG 14 days between bulls and heifers.

At d14, calves that were treated for illness in the first 14 days of life (n=9) had a lower body weight compared to calves that were not treated (treated: 41.0±1.4, not treated: 46.1±0.8 kg; $F_{1,30.0}$=10.1, P=0.003), but disease incidence did not affect ADG. Calves born in April had a greater ADG group (725.1±147.8 g/day) compared to calves born in February (447.0±101.3 g/day), March (334.3±71.3 g/day) and May (203.1±132.3 g/day; $F_{3,28.0}$=2.7, P=0.067), although the difference was not statistically significant. A significant effect of birth month was found on ADG 14 days (Feb: 211.3±60.5; Mar: 115.1±40.9; Apr: 382.8±70.8; May: 125.6±70.8 g/day, $F_{3,27.0}$=3.2, P=0.039).

5.3.2. Activity level

The final models used for LP hutch and LP group are shown in **Table 5.5**.

Table 5.5. Final models used to analyse LP hutch and LP group.

	Final model
LP hutch	*treatment + day (hutch) + breed + disease incidence (hutch) + milk type*
LP group	*treatment + day (group) +disease incidence (group) + month*

There were no significant differences in LP hutch between the treatment groups (I: 0.85±0.02, G: 0.85±0.02, K: 0.84±0.02; $F_{2,23.9}$=0.5, P=0.588) and the “day(s) in the hutch” ($F_{4,98.2}$=1.9, P=0.121). Both I and K calves decreased their LP from d2 to d5, whilst G calves increased their LP (**Figure 5.1**). Calves fed whole milk in the hutch had a significantly lower LP (0.83±0.01) compared to calves fed milk replacer (0.87±0.02, $F_{1,23.9}$=4.7, P=0.040). Breed and disease incidence also had no significant effects on the LP hutch (beef-cross: 0.86±0.01, dairy: 0.84±0.02, $F_{1,22.4}$=1.2, P=0.296; treated: 0.87±0.02, not treated: 0.83±0.01, $F_{1,25.2}$=3.7, P=0.067).

There were also no significant differences in LP group between the treatment groups

($F_{2,5.2}$=3.7, P=0.101) and the "day in the group pen" ($F_{1,40.5}$=0.6, P=0.433). However, a pairwise comparison showed that LP group d1 for G calves (0.83±0.02) was significantly higher than K calves (0.78±0.02, t=2.6, P=0.048), but not significantly different to I calves (0.79±0.02, t=1.7, P=0.142; **Figure 5.2**). There was also no difference in LP group between the disease incidence in the hutch ($F_{1,45.6}$<0.1, P=0.975) and observation month ($F_{3,24.9}$=2.0, P=0.143).

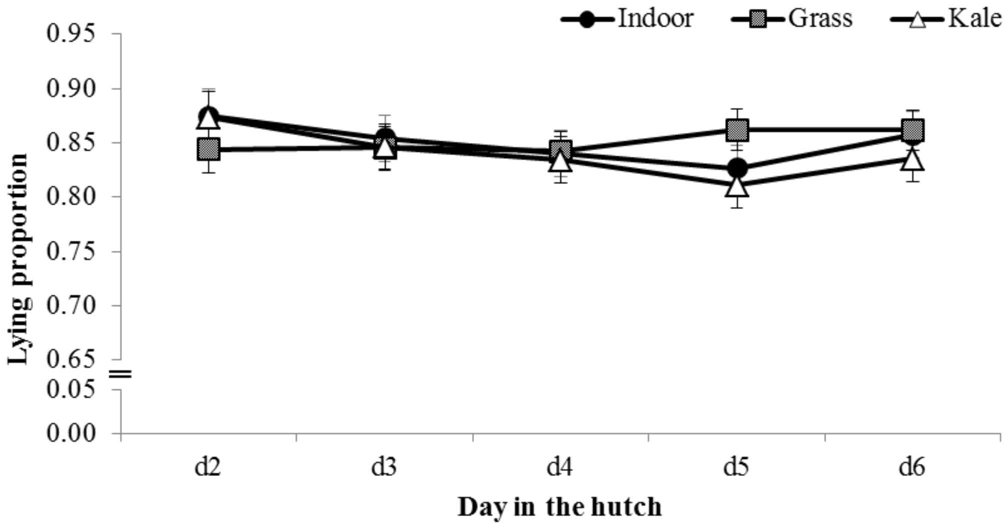

Figure 5.1. Lying proportion of calves in the hutch (d2-d6).

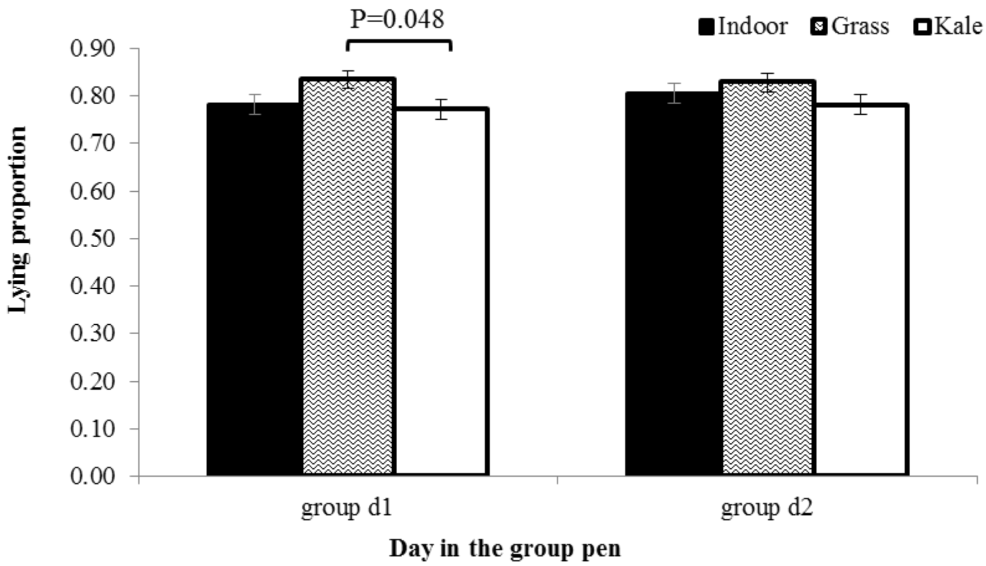

Figure 5.2. Lying proportion on the first day (group d1) and the second day (group d2) in the group pen.

5.3.3. Learning ability and ease of learning

Table 5.6 summarises the final models used to analyse training count and ease of training scores.

Table 5.6. Final models used to analyse training count and ease of training scores.

	Final model
Training count	*treatment + group size*
Ease of training score	
Willingness to enter the feeder	*treatment + disease incidence (hutch) + pen†*
Ease of finding a teat	*treatment + pen†*

†Models for ease of training score were adjusted for pen location.

A significant treatment effect was found on the training count ($F_{2,31.0}$=4.5, P=0.020). G calves required significantly fewer trainings (2.9±0.5) compared to I calves (4.2±0.5, t=2.9, P=0.007), and required fewer trainings compared to K calves (3.5±0.5, t=1.9, P=0.073), although this difference was not significant. The training count for K and I calves was not significantly different.

Calves introduced into a group with a larger group size required 0.10±0.03 more training for each unit increase in group size, $F_{1,31.0}$=10.4, P=0.003). Distributions of calves across the ease of learning scores are summarised in **Table 5.7**. Maternal treatment had no effect on the "willingness to enter the feeder" score (P=0.871) and the "ease of finding a teat" score (P=0.867). No effect of disease incidence was observed in the "willingness to enter the feeder" score (P=0.698). The training count until calves self-fed from the automatic milk feeder in the group pen had a significant effect on the growth in the first 14 days ($F_{1,27.0}$=6.4, P=0.018). Calves had a lower ADG d14 if they required more training until they self-fed (decrease by 62.1±24.6 g per unit increase in group size).

Table 5.7. Distribution of calves in each of the treatment groups for the “willingness to enter the feeder” score and the “ease of finding a teat” score. Odds Ratios and corresponding 95% CIs were reported as either the Indoor or Kale group as a reference level (Ref).

	Willingness to enter the feeder			**Ease of finding a teat**		
	Indoor	**Grass**	**Kale**	**Indoor**	**Grass**	**Kale**
0: No help	0	0	0	0	3	2
1: Easy	2	1	1	6	7	7
2: Not easy	6	7	5	1	2	0
3: Difficult	2	6	5	2	1	2
4: Very difficult	NA	NA	NA	1	1	0
Odds ratio [95% CIs]	Ref	2.4 [0.2, 35.1]	2.5 [0.1, 44.3]	Ref	0.4 [<0.1, 7.6]	0.3 [<0.1, 0.3]
	0.6 [0.1, 4.7]	0.9 [0.1, 6.1]	Ref	1.3 [0.2, 9.4]	1.0 [0.2, 6.7]	Ref

NA: not applicable as no category score 4 for this measure.

5.3.4. Reactions to a group environment

The final models for the behavioural reactions to a group environment are summarised in **Table 5.8**.

Table 5.8. Final models used for the analyses of behavioural reactions to a group environment.

	Final model
Latency	
walk	*treatment + disease (hutch) + group size + pen†*
run	*treatment + breed + group size + pen†*
explore	*treatment + (pen)*
enter the igloo shed	*treatment + month + human inside + human outside + pen†*
lie down	*treatment + breed + (pen)*
initiate social contact	*treatment + gender + month + human outside + pen†*
receive social contact	*treatment + gender + breed +human inside + human outside + pen†*
Duration	
stand inactive	*treatment + disease (hutch) + month + human inside*
walk	*treatment + gender + breed + group size + month + human outside*
run	*treatment + group size + month*
explore	*treatment + breed + month + human outside*
social interaction	*treatment + human inside + human outside*
straw yard	*treatment + group size + human inside + human outside*
igloo shed‡	*treatment + human inside + human outside*
Frequency	
walk	*treatment + gender + breed*
run	*treatment + group size + month*
initiate social contact	*treatment + month + human inside + human outside*
receive social contact	*treatment + breed + human inside + human outside*
explore	*treatment + breed*

†The final models for latency were adjusted for pen location.

‡The data were analysed following a square root transformation.

Latency

Table 5.9 summarises the median latencies of calves to perform walk, run, explore the pen, initiate, and receive social contact (calculated from the raw data) for the first time after introduction to the group pen. Not all calves lay down or entered the igloo shed within the observation period, and so the data were analysed using Cox regression, and the percentage of calves lying down and entering the igloo shed within the observation period are indicated in **Table 5.9**.

There was no significant difference between treatment groups in the likelihood of calves to start walking, running, exploring the group pen, first entering the igloo shed, initiating social contact with pen mates or receiving social contact from pen mates after the introduction to the group pen. A significant effect of treatment was found on the latency to lie down (*df*=2, P=0.047), where G calves were more likely to lie down within 30 minutes after the introduction to the group pen compared to I calves (HR=4.0 [0.9, 19.2]), and K calves (6.1 [1.1, 33.1]). No significant breed effect was observed on the latencies to perform any of the behaviours, but dairy calves tended to lie down quicker than beef-cross calves (HR=5.4 [1.1, 26.5], P=0.053). Compared to bull calves, heifer calves took significantly longer to receive social contact from pen mates (HR=0.3 [0.1, 0.7], P=0.005), but a gender effect was not observed on the latency to perform any of the other behaviours.

Calves started walking sooner as the group size increased (HR=1.3 [1.0, 1.6]), but group size did not affect the latencies to perform any of the other behaviours. Human presence significantly affected the latencies of calves to enter the igloo shed, but not the other behaviours. When a human was present inside the group pen, calves were more likely to enter the igloo shed (HR=10.3 [1.4, 73.0], P<0.001), compared to when a human was not inside the group pen. In contrast, when a human was present outside the group pen, calves were less likely to enter the igloo shed (HR=0.05, [0.008, 0.24], P=0.018) compared to when humans were not outside the group pen. Disease incidence in the hutch and observation month had no impact on the latencies of any of the behaviours.

Table 5.9. Median latencies of behaviours (interquartile values) calculated from the raw data, or the number of calves that performed the behaviour (%) during the 30 minutes of observation period after introduction to the group pen.

Behaviour	**Median (seconds) (Q1-Q3)**			**P-value**
	Indoor (I) n=10	**Grass (G) n=13**	**Kale (K) n=10**	
Walk	1.3 (0.0-49.1)	2.3 (0.1-8.0)	5.2 (2.2-17.4)	0.322
Run	43.3 (22.4-90.2)	31.6 (5.6-94.0)	34.6 (8.6-91.4)	0.798
Explore	6.9 (3.3-15.3)	9.8 (5.3-19.1)	15.7 (6.0-58.8)	0.283
Initiate social contact	44.9 (10.0-196.5)	50.0 (19.0-275.0)	51.0 (11.0-154.0)	0.664
Receive social contact	195.0 (59.0-313.0)	163.7 (73.9-499.8)	100.9 (38.4-215.6)	0.101
	N calves performed (%)			
Lie down*	3 (30.0)	7 (53.8)	2 (20.0)	**0.047**
Enter the igloo shed*	9 (90.0)	12 (92.3)	9 (90.0)	0.749

*Not all calves performed the behaviour “lie down” and “enter the igloo shed”.

Duration

There was no significant difference between the treatment groups in the times spent standing inactive, walking, running or exploring the group pen, nor was there any difference in the duration of time that calves spent in the igloo shed or the straw yard (**Table 5.10)**. However, a significant treatment effect was found on the time spent on social behaviour ($F_{2,26.8}$=3.5, P=0.045). G calves were more likely to sniff or lick pen mates, compared to K calves (P=0.014; **Table 5.10**).

A significant effect of observation month was observed on the duration of standing inactive ($F_{3,25.0}$=4.5, P=0.012), with calves standing inactive for a significantly longer time in May (15.6±1.8 min) compared to the other months (Feb: 8.9±1.8; Mar: 10.6±1.0; Apr: 9.2±2.0 min). The effect of month was not observed on the duration of walking ($F_{3,17.4}$=1.6, P=0.232), running ($F_{3,17.4}$=1.7, P=0.168), or exploring the group pen ($F_{3,25.0}$=2.6, P=0.077). Group size had no significant effect on the duration of walking ($F_{1,13.7}$=1.6, P=0.221) and running ($F_{1,13.7}$=0.1, P=0.754). There was a marginal effect of group size on the time spent in the straw yard, with calves spending 0.54±0.27 min less time in the straw yard for each unit increase in the group size ($F_{1,27.0}$=4.2, P=0.051). There was no breed effect on the duration of walking (beef-cross: 5.4±0.4, dairy: 4.4±0.8 min, $F_{1,22.5}$=1.7, P=0.204) and exploring the group pen (beef-cross: 9.0±0.6, dairy: 6.3±1.3 min, $F_{1,25.0}$=3.9, P=0.060). There were no significant differences in the duration of any behaviours measured associated with gender, disease incidence or human presence (inside and outside).

Frequency

There was no significant effect of treatment on the frequencies of any of the behaviours observed (**Table 5.10**). No significant overall effect of the three maternal treatments was observed on the frequency of social contact initiated by calves ($F_{2,25.0}$=2.3, P=0.124), but comparisons between two groups showed that G calves initiated significantly more frequent social contact with pen mates compared to K calves (t=2.3, P=0.044).

There was a significant effect of breed on the frequency of social contact received ($F_{1,27.0}$=7.9, P=0.009), where dairy calves received social contact from pen mates more frequently (21.3±3.7 time) compared to beef-cross calves (10.4±1.6 times). A significant breed effect was not observed in the frequency of any other behaviours, but beef-cross calves tended to explore the pen more frequently compared to dairy calves (beef-cross: 61.4±3.1, dairy: 44.0±8.2 times, $F_{1,29.0}$=4.0, P=0.057). There were no significant differences related to gender, observation month, group size, and human presence (inside/outside) in the frequency of any of the behaviours measured.

Table 5.10. Mean duration and frequencies (±SEM) of behaviours performed by calves during the 30 minutes of observation period after introduction to the group pen. Back-transformed means [95% CIs] were reported for the duration in the igloo shed.

	Indoor	**Grass**	**Kale**	W statistic	P-Value
Duration (minutes)					
Standing inactive	10.6±1.5	10.1±1.2	12.5±1.7	2.4	0.314
Walking	5.2±0.6	4.7±0.7	4.7±0.7	0.4	0.813
Running	2.1±0.4	1.5±0.4	1.8±0.4	5.1	0.168
Exploring	8.1±1.0	7.3±1.0	7.6±1.1	0.5	0.773
Social interaction	3.7±0.9	4.5±0.8	1.9±0.9	7.0	**0.048**
Straw yard	21.2±2.7	19.4±2.6	21.0±2.9	0.4	0.840
Igloo shed*	4.9 [0.9, 16.7]	7.1 [2.0, 29.8]	5.9 [1.3, 21.8]	0.5	0.796
Frequency (count/30min)					
Walking	58.2±5.3	47.8±5.3	51.3±5.8	2.7	0.270
Running	22.2±4.0	17.3±3.7	22.5±3.9	1.3	0.574
Exploring	56.7±5.8	45.9±5.8	55.6±6.2	3.2	0.225
Social contact initiated	27.2±4.7	31.3±4.6	19.3±5.3	4.6	0.124
Social contact received	15.5±2.6	18.7±2.8	13.3±2.9	3.3	0.207

* The data were analysed following a square root transformation.

5.4. Discussion

These results suggest that out-wintering pregnant heifers does not affect the birth weight of calves. However, on average, calves from all the treatment groups lost weight during the first week of life, and the average daily weight loss for G calves was the greatest. This resulted in significantly lower body weight of G calves at the point of introduction to the group pen (d7±1) compared to K calves. Moreover, the average daily weight gain of G calves in the group pen was the lowest, which resulted in G calves having a significantly lower daily gain for the first 14 days compared to I and K calves. Higher body weight at birth and weaning are associated with increased survival in dairy calves (Henderson et al., 2011). Although there was no statistically significant difference in birth weight and disease incidence in the hutch, the slowest growth rate, and lowest body weight of G calves suggest that maternal out-wintering on deferred grass could be disadvantageous in offspring survival.

It has been reported that prepartum maternal nutritional levels affect the body weight and postnatal growth of calves (Wu et al., 2006; Funston et al., 2010a). More specifically, literature in out-wintered beef cattle suggests that insufficient protein intake of the mother during late gestation may affect offspring post-weaning growth and productivity (Martin et al., 2007; Larson et al., 2008). For example, winter grazing of pregnant cows during late gestation with or without protein supplementation did not affect calf birth weight and average daily gain (Martin et al., 2007), but prepartum protein supplementation improved offspring weaning and pre-breeding weight as well as 205d adjusted body weight (Martin et al., 2007; Larson et al., 2008). Funston et al. (2010a) suggested in their review that prepartum protein supplementation in dams enhances the development of muscle fibres in the fetus, which potentially leads to getter postnatal growth of offspring.

The current study was part of a larger project looking at the out-wintering of heifers (Hargreaves et al. 2016, unpublished), of which only the calf work is presented in this thesis. However, some of the other data collected as part of the wider project might be helpful in interpreting the effect of maternal nutrition on calf weight. The data for pregnant heifers indicated that the level of maternal dietary protein was lowest in the I group and highest for the K group (**Table A.1.** in Appendix). The level of prepartum blood urea was higher in the K group than the G and I groups (**Table A.1.**), suggesting that K heifers had the highest protein intake during late pregnancy. The concentration of prepartum blood albumin in the I group was lower than the G and K groups, confirming that protein intake was the lowest in the I group.

However, prepartum body weight gain was greater in the I group than the K group (**Table A.2**). This could be attributed to a different housing system, where exposure to a cold winter environment required higher energy expenditure for K heifers compared to I heifers to support their metabolism and growing fetuses. Indeed, prepartum weight of I heifers was significantly higher than the out-wintered heifers when the outside temperature was lowest (-2.0 ˚C, **Figure A.1** in Appendix), and ground was covered by snow (Hargreaves et al., 2016 unpublished). In contrast, prepartum protein intake in G heifers was at a similar level as in I heifers, and prepartum weight gain of G heifers was also similar to I heifers (i.e. greater than K heifers). This may suggest that G heifers used the available energy for maintenance instead of fetal development, which may, in turn, have resulted in the lowest growth rate in G calves.

Keogh et al. (2009) investigated the effect of out-wintering pregnant dairy cows (winter grazing on kale, swede or perennial deferred grass) compared to cows kept indoors with grass silage and concentrate in the winter. Cows out-wintered on deferred grass lost weight and their body condition score decreased during the prepartum period, while cows in the indoor treatment gained weight. Furthermore, cows in the indoor system and out-wintered on kale or swede also improved their body condition. From the results of the current study and that of Keogh et al. (2009), it is possible that out-wintering pregnant heifers may have minimal effects when heifers are allowed to graze on kale, which could provide sufficient protein. However, out-wintering of pregnant heifers on deferred grass during late pregnancy may compromise the growth of heifers and/or fetal development.

There was no significant difference between the treatment groups in disease incidence. It has been reported that winter grazing of pregnant cows without protein supplementation resulted in more calves receiving treatment for bovine respiratory disease (Larson et al., 2008). It was not clear from the current study whether disease incidence was affected by maternal protein level, but the percentage of calves treated for diarrhoea or respiratory disease was higher in G calves compared to K calves (although this was not statistically significant). The percentage of calves requiring treatment was lowest in the I group, suggesting that disease incidence may have been affected by prenatal protein levels and the housing system. A larger sample size would be needed to investigate any statistical significance of this observation.

Calves in all treatment groups spent approximately 85 % of their time lying when they were kept in the hutch. In a semi-natural condition, calves hide in bushes in the first days of life, spending the majority of time lying when their mothers are away (Vitale et al., 1986). I and K calves decreased their lying proportion gradually from d2 to d5, but this was not observed in G calves. This could possibly suggest that G calves were less vigorous in the first week of life and required more rest. It has been reported that there is a positive correlation between resting time and growth in dairy calves (Mogensen et al., 1997; Hänninen et al., 2005). However, the higher lying proportion of G calves in the current study was not associated with better growth.

The lying proportion for I and K calves decreased to less than 80% after they were introduced to the group pen. Jensen et al. (1998) reported that calves confined in a small space increased their locomotor behaviour when introduced to a larger space. Decreased lying proportion in K and I calves may indicate their motivation for locomotor activity, as they spent more time standing and possibly engaged more in locomotor activity. In contrast, the lying proportion for G calves remained higher than 80% in the group pen, which may indicate that G calves were less motivated to perform locomotor activity. This is in agreement with a shorter latency of G calves to lie down during the first 30 minutes of observation period.

The behavioural reactions in a group environment with novel companions were observed for the first 30 minutes after introduction to the group pen. A significant difference was found in the social behaviour between the two out-wintered treatment groups. G calves made more frequent social contact and spent longer interacting with companions compared to K calves. Sniffing of companions can be considered an indication of social motivation, which can be seen from 2 days of age in dairy calves (Duve and Jensen, 2012). Therefore, it appears that G calves were more motivated to socialise with companions compared to K calves.

However, the current study only observed a single parameter of calf social behaviour when calves were first introduced to a novel social environment. More detailed observations of social behaviour such as proximity to neighbouring calves and behavioural synchrony would help assess whether G calves had a higher social motivation and a better social ability. It has been reported that social housing (i.e. physical contacts) from an earlier age could affect the development of social behaviour (Jensen and Larsen, 2014) and cognitive ability in dairy calves (Gaillard et al., 2014). No significant difference was found in social behaviour between calves born to indoor heifers (I) and born to out-wintered heifers (G, K), suggesting that the difference in the social behaviour could be attributed to maternal nutrition, and not maternal out-wintering.

There were no differences between the treatment groups in locomotor behaviours (walking, running), exploratory behaviours (sniffing/licking of objects), and the duration of standing inactive in the first 30 minutes after introduction to the group pen. It has been reported that maternal stress during pregnancy increases fear and anxiety-related behaviour in offspring (Fride and Weinstock, 1988; Dickerson et al., 2005; Murmu et al., 2006). In dairy cattle, longer durations of immobility, lower locomotor activity, longer latency to approach a novel object and less frequent interactions with the novel object are associated with fear (Boissy and Bouissou, 1995; Van Reenen et al., 2004). In the current study, there was no evidence that the maternal treatment affected fearfulness of offspring in reactions to a novel social environment.

The current study showed that calves born to heifers out-wintered on deferred grass required less training than H and K calves to self-feed from the automatic milk feeder. A delay in learning to use an automatic milk feeder has some undesirable consequences for farmers and calves, including extra labour for the former (Kung et al., 1997) and a lower milk intake in the latter (Fujiwara et al., 2014). The speed of learning can be affected by various factors such as the level of hunger and the adaptability animals exhibit when presented with a novel environment and novel companions. G calves lost most weight in the hutch, and so may have been hungrier than K and I calves. It has been shown in other animal species that rats were better at learning about resources (food) in a hungry state compared to a sated state. Therefore, a higher speed of learning to self-feed in G calves may have been driven by higher motivation for milk.

G calves were involved in more social contact in the first 30 minutes after introduction to the group pen, which may indicate that they were more social. Associations between better cognitive ability and social experience have been reported in dairy calves (Gaillard et al., 2014; Meagher et al., 2015). Therefore, higher social motivation in G calves might have helped them adapt quicker to a group environment, which might have helped them learn to use the automatic feeder faster than K and I calves as a consequence. The current study also found that calves requiring more training had a significantly lower average daily gain for the first 14 days of life. However, the average daily gain for the G group was still the lowest of the three treatment groups, which may highlight the importance of prenatal nutrition for calf growth.

In addition to the speed of learning to use the milk feeder, calves that were reluctant to enter the feeder would potentially increase the time spent by the farmer with the calves. The current study did not find significant differences between the treatment groups in the willingness to enter the feeder score. Regardless of treatment group, calves needed to be pushed from behind to enter the feeder. One third of calves struggled to find a teat, and the trainer needed to hold the head of calves to ensure drinking, a procedure that increased the labour time required by farmers. However, the current study did not find any differences between the three treatment groups in the “ease of finding a teat” score.

5.5. Conclusion

The current study indicates that out-wintering of pregnant heifers on deferred grass appears to have unfavourable effects on offspring growth and vigour, which might potentially affect their survival. Although this maternal treatment had an advantage in the speed of learning to use the automatic milk feeder, this did not compensate for the slower growth rate in the first 14 days of life. In contrast, growth, health, and behaviour of calves born to out-wintered heifers on kale were not different to calves born to indoor heifers. This might suggest that prenatal exposure to a cold environment might affect offspring performance if pregnant heifers were out-wintered on deferred grass. The behavioural observations conducted after calves were introduced to a social environment suggests that calves born to heifers on deferred grass made more frequent and longer social contact with companions. This study was conducted using a small sample size, and maternal stress and nutritional levels were not closely monitored. Therefore, it is not possible to conclude that it was maternal cold stress or insufficient nutritional intake that affected growth, activity levels and behaviour of offspring. However, the results from the current study would suggest that a maternal winter housing system and nutritional conditions during pregnancy may have played a role in the growth and behaviour of calves in the first two weeks of life. Further research with a larger sample size and more detailed observation is warranted to investigate the findings of this study further.

Chapter 6 :

General discussion and conclusions

6.1. Summary

6.1.1. Main findings

The overall aim of this project was to investigate the impact of management practices during late pregnancy on the performance of cows and their offspring. The first part of this project focused on developing an understanding of typical management practices during the dry period and identifying potential sources of stress for dry cows. A survey was conducted on UK dairy farms to build up a better picture of common management practices during the dry period and the pre-weaning period, which revealed some potential welfare issues for dry cows and pre-weaned calves.

The second part consisted of two experiments that investigated the effect that cow management practices during late pregnancy had on offspring health and behaviour. The first experiment investigated the effect of two differing stocking densities in the feeding and lying areas during the dry period on the health, physiology and behaviour of dry cows, and the health, growth and behaviour of their pre-weaned offspring (**Experiment 1**). The second experiment investigated the health, growth and behaviour of calves born to heifers that were either housed in the winter months or out-wintered on one of two differing grazing systems during late pregnancy (**Experiment 2**). The results from these two experiments suggest that there may be potential associations between maternal social environment during pregnancy and offspring social behaviour and reactions to weaning and disbudding, and between maternal nutritional levels during late pregnancy and offspring growth.

The main findings of the survey were as follows:

1) The majority of respondent farms managed their dry cows in dynamic social groups.
2) The majority of cows from respondent farms produced 10-20 kg/day of milk at dry-off, and were typically abruptly dried-off.
3) The majority of respondent farms reduced the quality of diet for their far-off dry cows.
4) UK dairy farmers have a good appreciation of recommended stocking densities for dry cows.
5) The majority of dairy farms separated calves from their dams within 24 hours after birth.
6) Pre-weaned calves were most likely fed 4.0L of milk per day and gradually weaned off milk around 8 weeks of age.
7) The close-up dry and early lactation periods were considered as the first and second most important periods in dairy production cycle, followed by young stock.

The hypotheses of Experiment 1 (**Chapter 3&4**):

- High stocking density during the dry period result in more frequent agonistic social interactions, altered feeding and lying behaviour, the activation of physiological stress response and negative energy balance in the cows.
- Maternal exposure to high stocking density during the dry period reduce offspring body weight, vigour, health and growth, and increase behavioural and physiological responses to challenges during the pre-weaning period

The main findings of the Experiment 1 were as follows:

1) High stocking density at the feed-face resulted in more aggressive interactions and shortened feeding times during the peak feeding period.
2) At high stocking density, cows were less likely to approach the feed-face and start feeding immediately after feed delivery.
3) The lying proportion and lying bout were not affected by stocking density.
4) Stocking density during the dry period did not affect prepartum metabolic function, levels of faecal glucocorticoid metabolites, or postpartum health and milk yield.
5) Maternal high stocking density during the dry period had no significant impact on calf birth weight, health, growth, passive immunity, neonatal vigour, reactions to human handling, learning ability, and exploratory and feeding behaviour in a group environment.
6) Calves born to cows in the high stocking density treatment initiated social contacts with companions more frequently in a group environment, and decreased their lying proportion during weaning compared to calves born to cows in the low stocking density treatment.
7) Maternal high stocking density reduced the behavioural reactions of healthy calves to a painful procedure (disbudding) and their expression of pain-related behaviour.

The hypotheses of Experiment 2 (**Chapter 5**):

- Maternal exposure to winter weather during pregnancy would reduce offspring body weight, health and growth, and increase behavioural responses to challenges in the first two weeks of life

The main findings of the Experiment 2 were as follows:

1) Out-wintering pregnant heifers on deferred grass resulted in the lowest growth rate of offspring, whilst these calves learned to use an automatic milk feeder the fastest.
2) Out-wintering on kale treatment had no disadvantageous effects on offspring growth, health and behaviour compared to indoor treatment.

6.1.2. Novelty of this project and limitations

6.1.2.1. Survey of dry cow and pre-weaned calf management practices

The main aim of this survey was to understand the typical experiences of dry cows and pre-weaned calves on UK dairy farms, which have not been previously investigated. There have been surveys that investigated management practices for lactating cows (e.g. March et al., 2014) and dry cows (e.g. Bertulat et al., 2015). The survey by Bertulat et al. (2015) investigated dry cow management practices in Northern Germany, but the survey questions mainly focused on dry-off procedures. There are surveys on dairy calf management practices (e.g. Vasseur et al., 2010; Staněk et al., 2014), but these were conducted outside the UK.

The information obtained from the survey provided a clear picture of common dry cow and pre-weaned calf management practices in the UK. Dry cow management procedures relating to welfare concerns included an abrupt dry-off of high yielding cows, dynamic social groups for dry cows and abrupt changes in diet from a high-energy lactating ration to a low-energy and high-fibre dry ration. Regarding pre-weaned calf management, early separation of newborn calves from dams and restricted milk feeding for pre-weaned calves appears to be common practice. However, it is worth noting that the majority of UK farms have a good awareness of the recommended stocking density of the feeding and lying areas for dry cows. The question about dairy producers' views on the dairy production cycle has revealed that the majority of respondents considered close-up dry cows and young stock as most important. This novel information should help understand farmers' perception towards the welfare of dry cows and pre-weaned calves.

Only the online version of the survey was available in the beginning, and the survey was mainly promoted via social media. The number of responses obtained from the online version was quite small (n=27), probably because social media only attracted computer literate farmers or farmers with easy access to the computer. Therefore, the paper version of the survey was prepared and several attempts were made to obtain the personal addresses of UK dairy farmers to distribute widely. Royal Associations of British Dairy Farmers (RABDF) agreed to distribute the paper version to their members, which resulted in the majority of respondent farmers being members of RABDF (n=106).

DEFRA (2015) reported that almost half (48%) of dairy holdings in the UK had less than 50 cows, which accounted for only 4% of dairy cows. In contrast, all respondent farms of the current survey had at least 50 cows. This may have contributed to the larger average herd size of respondent farms than the UK average. Nevertheless, the information from these farms would be more relevant to this survey compared to smaller farms, and the cows held on these farms would be a valid representation of the majority of cows in the UK.

6.1.2.2. ***Experiment 1****– high stocking density during the dry period*

The survey of UK dairy farmers found that dry cows were more likely to be managed in dynamic social groups than stable social groups. This suggests that cows may experience social stress during the dry period due to frequent regrouping. It has been reported that the effects of social regrouping such as competition at the feed-face and altered feeding activity could be worsened by increased stocking densities (Talebi et al., 2014). Therefore, the current study (**Chapter 3**) investigated the impact of two differing stocking densities at the feed-face and the lying area on cow behaviour and welfare. The experimental design for the high stocking density treatment was based on the minimum space allowance requirements of the UK Red Tractor Assurance for Farms - Dairy Standards (2014). Cows in the current study were also managed in dynamic social structures, as this was a routine social grouping system on the experimental farm (i.e. weekly dry-off and movement from far-off to close-up management groups).

There have been studies that investigated the effect of stocking density during the prepartum period (Holstein cows: Proudfoot et al., 2009; Huzzey et al., 2012; Fustini et al., 2017; Jersey cows: Lobeck-Luchterhand et al., 2015; Silva et al., 2016, 2014). The experimental settings of these studies were slightly different (**Table 1.1**) and the treatment period was shorter (30 days or less) compared to the current experiment (60±6SD days). However, the overall results from the current experiment were in agreement with the previous findings, confirming that high stocking density increased agonistic interactions at the feed-face and altered feeding activity. The current study also agreed with the findings of Huzzey et al. (2006) who reported that the yoke feed barrier system reduced the competition for feed at high stocking density. The current study also suggested that multiparous cows were better at coping with the crowded feeding area, which was also reported by Proudfoot et al. (2009).

There were some discrepancies between the results from the current and previous studies regarding the metabolic and physiological parameters (Huzzey et al., 2012; Lobeck-Luchterhand et al., 2015). As discussed in **Chapter 3**, these discrepancies could be attributable to different experimental settings and experimental periods. However, large individual variations found in the current study may have also played a role. The current experiment used cows that entered the dry period between November and June 2015, and new cows entered the treatment groups almost every week. Additionally, non-focal cows (severe lameness, longer dry period, targeted for culling) were included in the dry cow group, which also changed over the experiment. This approach was inevitable as there were not always sufficient numbers of dry cows available for the experiment, and spaces for non-lactating cows were limited. The dynamic social structures in the current experiment may have increased individual variations within the group, but may also have represented a typical environment for dry cows on commercial farms.

6.1.2.3. ***Experiment 1****- calf outcomes*

Rodent studies have shown that maternal social stress during pregnancy can be associated with lower birth weight, enhanced responsiveness of the HPA axis, increased anxiety-related behaviour and impaired social behaviour in offspring (Brunton, 2013). To my knowledge, this is the first study that investigated associations between stocking density during the dry period and offspring growth, health and behaviour in dairy cows. The current study (**Chapter 4**) found no evidence that maternal high stocking density during the dry period affected offspring birth weight, growth and neonatal vigour. In contrast, disease incidence had a greater impact on pre-weaning growth and activity levels, highlighting the importance of disease control for improved growth in pre-weaned calves.

Based on studies on laboratory rodents and other farm animals (Brunton, 2013; Otten et al., 2015), it was hypothesised that maternal social stress during the dry period might affect offspring response to aversive conditions such as an exposure to a fear-eliciting environment, weaning and painful procedure. Fearfulness in dairy calves can be assessed using various behavioural tests, including an open field test or novel object test (de Passillé et al., 1995; Meagher et al., 2016). Animal's social motivation can be assessed using a social isolation test or a social preference test (Færevik et al., 2006). During these behavioural tests, animals are normally presented in a test field alone (or with a companion animal for the social preference test), and their behaviour is monitored for 5-10 minutes.

Due to time and labour constraints, the current experiment did not use these behavioural tests. Instead, the behavioural response of calves to procedures that are normally conducted on the experimental farm was assessed. This approach allowed for assessing reactions of calves to a commercially relevant environment but made it difficult to interpret the results, as it could not distinguish between different factors affecting calf behaviour. Additionally, due to limited availabilities of automatic milk feeders and group pens, calves in the current experiment were grouped with non-experimental calves. This might have affected how individual calves behaved in a group environment and created large individual variations.

No effect of treatment was observed in the behavioural parameters related to learning ability, feeding behaviour, exploratory behaviour and human handling. This was not surprising considering the lack of difference in maternal cortisol level between the treatment groups.

Prenatal exposure to excess maternal glucocorticoids is a potential mechanism affecting fetal brain development and offspring behaviour (Charil et al., 2010). Nonetheless, significant treatment effects were found on social behaviour and the reactivity of calves to weaning and disbudding. The results indicated that H calves were more motivated to socialise, but there was a tendency for L calves to accept social contact from companions. H calves spent less time lying and stayed closer to the feeders during weaning, but this was not observed in L calves. The reactivity of calves to disbudding and their pain-related behaviours were reduced not only by their health status, but also by maternal high stocking density. These outcomes could support the hypothesis that maternal high stocking density might affect behavioural reactions of calves in aversive conditions.

Kranendonk et al. (2007) reported that there were associations between maternal social ranks and offspring body weight and behaviour, even though there was no difference in the levels of maternal salivary cortisol. Piglets born to high-ranking sows were more active and vocalised more during the novel object test, compared to piglets born to low-ranking sows. Similarly, Ison et al. (2010) found that gilts with more skin lesions (due to receiving more attacks from sows) after social mixing with sows during the mid-gestation period gave birth to less active and less aggressive piglets, despite the lack of correlations between maternal cortisol levels and piglet's behaviour. These studies demonstrated the effect of maternal stressful experiences during pregnancy on offspring behaviour without any associated elevation of maternal stress hormones. Together with the results from the current study, these associations could be explained by inherited personality traits, but it could also be argued that there were other factors mediating maternal-offspring relationships.

6.1.2.4. ***Experiment 2*** *- effect of maternal out-wintering on offspring*

The second experiment used calves born to heifers out-wintered during late pregnancy (**Chapter 5**). This is also, to my knowledge, the first study in dairy cattle that investigated the effect of out-wintering pregnant heifers on offspring growth, health and behaviour. Studies on beef cattle investigated the effect of maternal out-wintering on offspring development (e.g. Martin et al., 2007; Larson et al., 2008; Funston et al., 2010b), but the main interests of these studies were calf growth and not behaviour. Pre-weaned beef calves are normally reared by dams, so their postnatal experience would be very different and less challenging compared to that of calves on dairy farms. The outcomes of this experiment suggested that maternal nutrition levels may be of critical importance to offspring growth, and are associated with the speed of learning to use an automatic milk feeder, which itself also affects growth.

The main interest of the out-wintering project was to investigate the economic feasibility of this management practice, and so there was no behavioural or physiological data of heifers related to stress. Other studies have suggested that exposure to harsh winter weather induces physiological stress responses in dairy cows (Tucker et al., 2007; Webster et al., 2008). It would be interesting to investigate the stress levels of out-wintered pregnant heifers and its association with offspring growth and behaviour. Such research would be of benefit in implementing a better out-wintering management strategy to improve the performance of both cows and calves.

6.2. Future research

6.2.1. Possible stressors for pregnant cows

The current cow experiment (**Chapter 3**) focused on social stress in dry cows. However, as discussed in **Chapter 1**, various factors associated with management practices could be stressful to pregnant dairy cows. The results from the survey have suggested that cows around dry-off experience changes in routine, such as abrupt cessation of milking, changes in diet, housing system and social environment. The abrupt dry-off would be a stressful event for high yielding cows in particular. From the current cow experiment, the sharpest increase in the concentrations of faecal glucocorticoid metabolites was observed in the first week after dry-off, which may have reflected stressful experiences just after dry-off. Efforts for mitigating the stress of cows around dry-off could have implications for improved dry cow welfare and fetal development.

6.2.2. Investigation of cow-calf relationship

The first experiment (**Chapter 3 and 4**) primarily aimed to investigate the effect of two differing stocking densities on cow and calf performance. Therefore, the outcomes were evaluated by group comparisons. However, it has been reported in various animal species that there are individual variations in responses to stress (Koolhaas et al., 2011), which can be characterised by the personality, stress coping style and social status of individual animals (Proudfoot et al., 2012). This implies that the experiences of cows in the high stocking density group could have varied between individual cows, and their offspring might have been affected accordingly.

Studies on farm animals have demonstrated that the social status of animals affects their reactions to challenging social environments (Mendl et al., 1992; Dwyer and Bornett, 2004; Val-Laillet et al., 2008a). Competitive behaviour at the feed-face is often used to classify social rank (Val-Laillet et al., 2008a) or to calculate dominance index (DI: Kondo and

Hurnik, 1990; Galindo and Broom, 2000) in dairy cows. Heifers are normally in a lower dominance rank when they are grouped with adult cows (González et al., 2003). Therefore, pregnant heifers are likely to experience social stress due to the dynamic social structure of dry cow groups and/or high stocking densities.

Val-Laillet et al. (2008a) found that cows that spent more time at the feed-face during the peak feeding period were likely to be dominant, whilst subordinate cows were forced to change their feeding behaviour. Additionally, lower-ranked cows were more likely to initiate displacements to gain access to feed compared to higher-ranked cows. In contrast, Lobeck-Luchterhand et al. (2015) reported that there was no effect of social rank on feeding and lying behaviour when cows were understocked (more than one feed yoke and cubicle per cow). They also reported that middle-ranked cows (DI=0.4-0.6) spent more time feeding during the peak feeding period compared to high (DI>0.6) or low (DI<0.4) ranked cows at higher stocking density (one feed yoke and cubicle per cow).

The dominance indices indicate individual success in displacements at the feed-face (Val-Laillet et al., 2008a), meaning that those cows that never appeared at the feed-face during the peak feeding period could be classified as lowest rank. However, a study in pigs (Mendl et al., 1992) found that pigs in the middle rank were often involved in aggressive interactions with little success, and showed higher levels of salivary cortisol compared to lower-ranked pigs that were not involved in aggressive interactions. This suggests that the stress levels of individual animals may not necessarily be directly related to their social rank, but rather may be associated with their behavioural responses to competitive environments.

Investigating the individual social status of cows from the current study and linking them with their measured behavioural and physiological parameters would help identify cows that were most susceptible to high stocking density. Furthermore, investigating the relationships between maternal factors such as social status and parity and offspring outcome could reveal stronger mother-offspring links, which might not otherwise have been detected using a group comparison.

6.3. Final conclusion

This study confirmed that high stocking density during the dry period increased competition at the feed-face in dry cows, and indicated that this did not affect offspring birth weight, growth and health. The majority of behavioural outcomes are not associated with maternal high stocking density. Potential effects of maternal treatment were found on social behaviour in the group environment and reactions to weaning, although these effects were not consistent with other outcomes. Moreover, the behavioural reactions of calves to disbudding and pain-related behaviour were altered by maternal treatment and the health condition of calves. Out-wintering pregnant heifers on deferred grass resulted in the lowest offspring growth rate, but enhanced the ability to find a novel feed source. Out-wintering pregnant heifers on kale showed no detrimental effects on offspring compared to indoor heifers. The results from the out-wintered project indicate that maternal nutrition levels had a greater impact on offspring than winter housing systems.

The current study has shown potential associations between maternal experiences during late pregnancy and offspring growth and behaviour, although further investigations are worthwhile to identify the underlying mechanisms. Future studies also need to consider social rank, parity and individual differences in stress response when designing an experiment. This would help evaluate maternal stress more accurately, and possibly detect stronger links between maternal stressful experience and offspring performance. Such research could lead to improved, less stressful, management practices for cows during late pregnancy, which could not only ensure the welfare and productivity of cows, but also promote the performance of replacement heifers and ultimately maximise future profitability.

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Appendix

Table A.1. The calculated mean diet of heifers kept indoors in winter (Indoor) with grass silage, out-wintered on perennial ryegrass (Grass) with grass silage and concentrates, and out-wintered on kale (Kale) with grass silage.

	Indoor	**Grass**	**Kale**
Dry Matter (g/kg)	448±51	202±14	193±7
Crude Protein (g/kg)	143±10	163±2	172±7
MAD Fibre* (g/kg)	443±80	469±20	469±25

*MAD Fibre: Modified Acid Detergent fibre

Table A.2. Mean prepartum dry matter intake (DMI), weight gain, body condition score (BCS), mean prepartum blood metabolite concentrations for urea (mmol/l), β-hydroxybutyric acid (BHB: mmol/l), albumin (g/l) and non-esterified fatty acid (NEFA: μmol/l) of heifers kept indoors in winter (Indoor) with grass silage and concentrates, out-wintered on perennial ryegrass (Grass) with grass silage, and out-wintered on kale (Kale) with grass silage.

	Indoor	**Kale**	**Grass**	**P-value**
Days on treatment	74	79	73	0.77
DMI (kg DM/cow/day)	7.4	7.6	8.1	-
Initial weight (kg)	563	559	562	0.96
End weight (kg)	642	612	613	0.10
Weight gain (kg/cow/day)	0.94	0.73	0.69	0.16
Initial BCS	2.63	2.34	2.53	0.004
End BCS	2.56	2.37	2.48	0.07
BCS change	-0.07	-0.05	0.03	0.08
Urea (mmol/l)	3.48	4.10	3.41	0.002
BHB (mmol/l)	0.55	0.36	0.41	<0.001
Albumin (g/l)	35.8	37.1	37.1	0.01
NEFA (μmol/l)	251	347	355	0.16

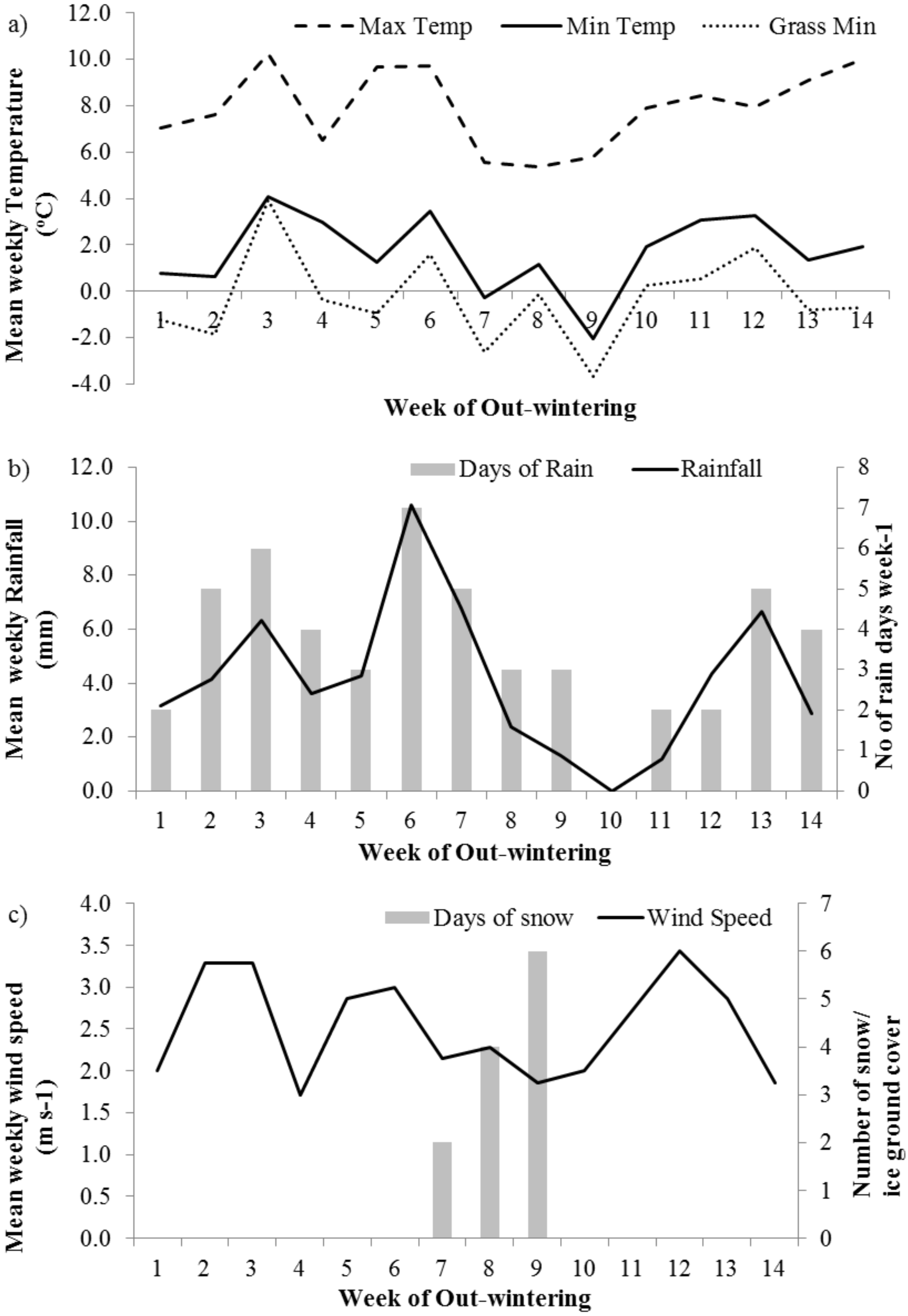

Figure A.1. Weekly mean meteorological data for Crichton Royal Farm for a) maximum air temperature (Max Temp: ˚C) minimum air temperature (Min Temp: ˚C), grass temperature minimum (Grass Min: ˚C), b) mean rainfall (mm) and days of rain (over 1mm recorded) and c) wind speed (m s^{-1}) with days of snow or ice ground coverage. (Data from Hargreaves et al., 2016, unpublished).

Survey of dry cow and pre-weaned calf management practices on UK dairy farms – distributed in April 2015

Dry Cow and Calf Management Survey

As part of a research project at Scotland's Rural College and the University of Edinburgh Veterinary School, we would be very grateful if you would complete this questionnaire. This will take 5 to 10 minutes, and you will be entered into <u>a prize draw to win £100</u>.

This project will investigate the management of dairy cows and the questionnaire focuses on the management of dry cows and pre-weaning calves.

<u>All responses are confidential</u> and the information you provide will be used to improve farm profitability and cow/calf health and welfare.

General information about your farm

Q1 How many of the following types of cow do you have in total?

All lactating cows/heifers ☐

Dry cows ☐

First lactating heifers ☐

Non-milking heifers (>6 months) ☐

Q2 What is the total annual milk sales on your farm?

☐ *litres*

Q3 What is the main breed of cow in your milking herd?

☐

Q4 What is your dairy replacement policy?

Breed all of your own.. ☐

Buy in some.. ☐

Buy in all.. ☐

Q5 Please **tick** which months of the year you have cows calving, and please **circle the single month** of the year when you usually have most cows calving.

Jan	☐	*May*	☐	*Sep*	☐
Feb	☐	*Jun*	☐	*Oct*	☐
Mar	☐	*Jul*	☐	*Nov*	☐
Apr	☐	*Aug*	☐	*Dec*	☐

Q6 Which group(s) of **dry** cows do you usually have on the farm?

- *Only a single group for dry cows* ☐
- *"Far-off" and "Close-up" dry cows* ☐
- *"Fat cow" group for over-conditioned dry cows* ☐
- *Other - please specify*

Q7 When do you mix pregnant maiden heifers/first lactation cows with adult cows?

- *Before first calving (during late pregnancy, mixed with adult dry cows)* ☐
- *After calving (when milking starts)* ☐
- *Maiden heifers/first lactation cows are kept in different group from adult cows* ☐

Q8 Do your **dry** cows remain in the same social group (no addition of new dry cows) during the dry period?

- *Yes, always* ☐
- *Yes, when possible* ☐
- *No, cows are added to group when they are dried off* ☐

Q9 Before the peak calving month (when you have the largest number of dry cows), what is the **maximum** number of cows in each of the following management groups? Please put the numbers where applicable.

Single dry cow group	
Far-off group	
Close-up group	
Fat cow group	
Other - please specify each group	

Drying off process

Q10 How long before their expected calving date are your cows typically dried off?

☐ ***days*** *before the expected calving date*

Q11 Which of the following is part of your drying off procedure?
Please tick all that apply.

- *1. Antibiotic tubes* .... ☐
- *2. Internal teat sealant* .... ☐
- *3. External teat sealant* .... ☐
- *4. Stop milking suddenly* .... ☐
- *5. Milking once a day*..............*go to Q11a* ☐
- *6. Milking every other day*........*go to Q11a* ☐

Q11a If you ticked 5 or 6 in Q11, for how many days do you normally continue this milking schedule?

☐ *day(s)*

Q12 In the last 12 months, please estimate how many cows fell into the following categories of milk production at drying off?

- *Less than 10 kg/day* ☐
- *10 kg to 20 kg/day* ☐
- *More than 20 kg/day* ☐

Q13 Do you manage your cows' diet to reduce milk production before drying off?

- *Yes, for all cows* .... ☐
- *Yes, only for high production cows*............ ☐
- *No*................................. *please go to Q14* ☐

Q13a Which of the followings are applicable to your procedure? Please tick all that apply.

- *Reduce the quantity of milking cow ration* . ☐
- *Change the type of silage but do not change the quantity* .... ☐
- *Change the type of silage and reduce the quantity* .... ☐
- *Reduce the quantity of concentrates* .... ☐
- *Stop concentrates*...................................... ☐
- *Add hay or straw to silage* .... ☐
- *Feed straw or hay* ***only*** .... ☐
- *Reduce water intake*.................................. ☐
- *Other -please specify*

☐

Q13b For how long do you usually apply this dietary management practice?

- *1 - 2 days*.. ☐
- *3 - 4 days*.. ☐
- *5 - 6 days*.. ☐
- *7 days or more*... ☐

Q14 Are your cows routinely foot trimmed **around drying off**?

- *Yes, before drying off* .... ☐
- *Yes, on the day of drying off*...................... ☐
- *Yes, after drying off* .... ☐
- *No, not around drying off*........................... ☐

Feeding

Q15 Which of the following feed do you give your cows in each stage of the production cycle? Please tick all that apply.

	Straw	*Hay*	*Grass silage*	*Whole crop/ Arable silage*	*Maize silage*	*Cereals, Starch/Other source of energy*	*Concentrates /Other source of protein*	*Mineral/ Vitamin supplement*
Late lactation	☐	☐	☐	☐	☐	☐	☐	☐
Far-off dry	☐	☐	☐	☐	☐	☐	☐	☐
Close-up dry	☐	☐	☐	☐	☐	☐	☐	☐
Calving	☐	☐	☐	☐	☐	☐	☐	☐

Q16 How do you usually feed concentrate or non-forage supplements to your dry cows?

	On top of silage/forage	*Mixed with silage/forage*	*Feed separately*	*Other*
Late lactation	☐	☐	☐	☐
Far-off dry	☐	☐	☐	☐
Close-up dry	☐	☐	☐	☐
Calving	☐	☐	☐	☐

Q17 Can all your **dry** cows feed at the same time after fresh feed is delivered?

Yes .... ☐ *No, not all of them can feed at the same time* .... ☐

Q18 How often do you deliver fresh feed to **dry** cows in the following groups? Please tick where applicable.

	Twice daily	*Once daily*	*Every second day*	*Less than every second day*
Single dry cow group	☐	☐	☐	☐
Far-off group	☐	☐	☐	☐
Close-up group	☐	☐	☐	☐
Fat cow group	☐	☐	☐	☐
Other dry group(s)	☐	☐	☐	☐

Q19 How often do you push feed up for **dry** cows in the following groups? Please tick where applicable.

	Three or more times a day	*Twice daily*	*Once Daily*	*Never*	*No need because of the feed bunk design*
Single dry cow group	☐	☐	☐	☐	☐
Far-off group	☐	☐	☐	☐	☐
Close-up group	☐	☐	☐	☐	☐
Fat-cow group	☐	☐	☐	☐	☐
Other dry group(s)	☐	☐	☐	☐	☐

Housing

Q20 **In summer**, which type of system is used for your cows during the following stages of the production cycle? Please tick all that apply.
<u>If cows are kept both inside and outside</u>, please indicate (by ticking the appropriate box) whether cows are free to move in and out, or whether are moved by farm staff at set times.

	Inside Cubicles	*Inside Straw/ Sand yard*	*Inside Other*	*Pasture/ Paddock*	*Wood chip/ No grazing*	*Cows are free to move*	*Cows are deliberately moved*
Late lactation	☐	☐	☐	☐	☐	☐	☐
Far-off dry	☐	☐	☐	☐	☐	☐	☐
Close-up dry	☐	☐	☐	☐	☐	☐	☐
Calving	☐	☐	☐	☐	☐	☐	☐

Q21 **In winter**, which type of system is used for your cows during the following stages of the production cycle? Please tick all that apply.
<u>If cows are kept both inside and outside</u>, please indicate (by ticking the appropriate box) whether cows are free to move in and out, or whether are moved by farm staff at set times.

	Inside Cubicles	*Inside Straw/ Sand yard*	*Inside Other*	*Pasture/ Paddock*	*Wood chip/ No grazing*	*Cows are free to move*	*Cows are deliberately moved*
Late lactation	☐	☐	☐	☐	☐	☐	☐
Far-off dry	☐	☐	☐	☐	☐	☐	☐
Close-up dry	☐	☐	☐	☐	☐	☐	☐
Calving	☐	☐	☐	☐	☐	☐	☐

Q22 Which one of the pictures do you think is the appropriate stocking density of a straw yard for dry cows?




☐ ☐ ☐ ☐

Q23 What do you think is the appropriate number of dry cows in a shed with <u>100 cubicles</u>?

105 cows	*100 cows*	*95 cows*	*90 cows*
☐	☐	☐	☐

Calf management

Q24 When do you separate calves from their mothers?

- *Less than 6 hours* ☐
- *6 hours to 24 hours* ☐
- *1 day to 4 days* ☐
- *5 days or more* ☐

Q25 Which of the followings best describe your housing for calves during their milk feeding period?

- *Individual pen (crate or hutch) from birth until weaning ....please go to Q26* ☐
- *Individual pen from birth to certain days of age then group pen until weaning ....* ☐
- *Group pen from around birth until weaning ....* ☐
- *Other - please specify*

Q25a From what age are your calves in group housing?

from ______ *days of age*

Q25b What is the average number of calves in your calf groups?

______

Q26 In the week before weaning begins, how much milk do you feed your calves?

- *4 litres or less/day* ☐
- *5 - 7 litres/day* ☐
- *8 litres or more/day* ☐
- *Ad libitum (milk is always available)* ☐

Q27 What is the average age of your calves at weaning ?

______ *weeks of age*

Q28 Over how many days does weaning usually take place?

over ______ *days*

Q29 When you use the following methods, at what age do you have your calves dehorned?

Caustic paste ______ *week(s) old*

Hot iron ______ *weeks old*

Q30 Do you use local anaesthesia (e.g. Adrenocaine, Willcaine, Lignocaine) before dehorning?

Yes ☐ *No* ☐

Information about you

Q31 What is your role on the farm?

- *Owner* ☐
- *Family member* ☐
- *Manager* ☐
- *Employed stock person* ☐

Q32 What is your sex?

- *Male* ☐
- *Female* ☐

Q33 What is your age range?

- *16 to 24* ☐
- *25 to 44* ☐
- *45 to 64* ☐
- *65+* ☐

Q34 How long have you been managing dairy cattle?

- *Less than 2 years* ☐
- *2 -5 years* ☐
- *6-10 years* ☐
- *11-20 years* ☐
- *More than 20 years* ☐

Q35 In your opinion, what are the **three most important periods** among the following five periods in dairy cow management? Please rank first, second and third, with 1 being the most important.

- *Young stock* ☐
- *Fresh calver/Early lactation* ☐
- *Mid to late lactation* ☐
- *Far-off dry period* ☐
- *Close-up dry period* ☐

Thank you for your time!!

If you would like to be included in a prize draw, please write your email address here.

☐

Please return this questionnaire to us in the envelope provided.

If you have any questions about this survey please contact

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